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Electrophoretic behaviour and infrared spectra of dihydroxyboryl compounds in aqueous di- and tricarboxylic acids: paper electrophoresis as a tool for determining the chemical states of a substance in solution

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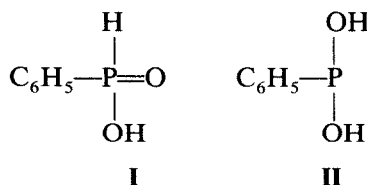
Abstract

p-Dihydroxyborylphenylalanine behaved as an anion in aqueous 0.1 mol dm⁻³ oxalic acid and 0.1 mol dm⁻³ citric acid in paper electrophoresis, but as a cation in aqueous 0.1 mol dm⁻³ fumaric, malic, tartaric, succinic, maleic and malonic acid. A similar electrophoretic behaviour was observed for boric acid. These results indicate that dihydroxyboryl compounds form complexes with oxalic and citric acid. The complex formation was confirmed by the infrared spectra of boric acid in the two aqueous carboxylic acids. The spectral behaviour was fully consistent with the migration behaviour in paper electrophoresis. It was confirmed that paper electrophoresis is a useful technique for determining the chemical states of a compound in solution.

1. Introduction

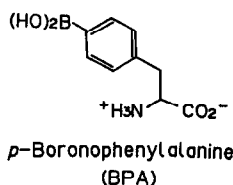
Electrophoresis is a useful technique for separating ionizable substances in solution. In electrophoresis, the mobility of a migrant depends on its net charge and apparent molecular mass. The net charge depends on both the p*K*_a value of the migrant and the pH of the supporting solution. The apparent molecular mass is influenced by the association of a migrant molecule with other molecules such as solute and solvent molecules. In principle, electrophoresis is useful for studying the chemical nature of a compound in solution [1,2]. Using electropho-

retic data, stability constants of chelate compounds [3] and dissociation constants of acids [4] have been determined. Elucidation of the chemical form of a compound is also possible. We observed that so-called phenylphosphonous acid (I) had two dissociable protons in aqueous solution, by analysing the mobility-pH curve in electrophoresis, [5] and assigned the chemical form of phenylphosphonic acid to phenylphosphinic acid (II) in aqueous solution.



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On the basis of the above, we adopted paper electrophoresis for studying the chemical nature of *p*-dihydroxyborylphenylalanine (*p*-boronophenylalanine; BPA) in aqueous solution [6]. BPA has been used clinically in the boron neutron capture therapy (BNCT) for malignant melanoma as a boron carrier [7], and a knowledge of the solution chemistry of medicines is essential in pharmacokinetics.



In the course of previous studies, we noticed that BPA behaved as an anion in aqueous 0.05 mol dm⁻³ oxalic acid [8]. Recently we preliminarily showed that BPA behaved as an anion in aqueous 0.1 mol dm⁻³ citric acid, but as a cation in aqueous 0.1 mol dm⁻³ fumaric, malic, tartaric, succinic, maleic and malonic acid [9]. The pH values of the aqueous carboxylic acid solutions are estimated to be <3. In such acidic solutions, BPA should be a cation owing to the protonated amino group (NH₃⁺). Hence we proposed that BPA formed a negatively charged complex with oxalic or citric acid.

Many workers have studied the complexation of dihydroxyboryl compounds (DHBCs) with *cis*-diols [10], and the resulting complexes have been utilized in affinity chromatography especially for the purification of diverse biomolecules [11]. Although studies on the complexation of DHBC with di- and/or polycarboxylic acids are limited, the complexation reactions of boric acids with some carboxylic acids such as oxalic [12] and tartaric [13] acid have been reported.

To obtain more evidence for complexation, we tried to elucidate the chemical form of the complex by infrared spectrometry, which is a useful technique for investigating solute–solute [14] and solute–solvent [15,16] interactions in solution. As BPA was scarcely soluble in water, we chose orthoboric acid as a candidate of DHBCs. The spectral behaviour was fully con-

sistent with the migration behaviour in electrophoresis. This means that paper electrophoresis is a useful technique for determining the chemical states of a substance in solution.

2. Experimental

2.1. Reagent and materials

Boric acid, oxalic acid dihydrate, citric acid monohydrate, malonic acid, succinic acid and maleic acid of guaranteed grade and oxalate pH standard solution (pH 1.68 at 25°C) were purchased from Wako (Osaka, Japan). BPA (¹⁰B-enriched) was supplied by Eagle Pitcher Research Laboratory (Miami, FL, USA). Ion-exchanged water was used after distillation. Toyoroshi No. 51A filter-paper (40 × 1 cm) was used as a support in electrophoresis.

2.2. Electrophoresis

The apparatus and procedures have been described previously [1,2,6]. The supporting solutions were aqueous 0.1 mol dm⁻³ oxalic, citric, fumaric, malic, tartaric, succinic, maleic and malonic acid. A 5-μl volume of sample (BPA and orthoboric acid) solution (about 5 · 10⁻³ mol m⁻³) or picric acid (5 · 10⁻³ mol dm⁻³) (the standard migrant) was spotted at three different positions on a paper strip (A, 5 cm to the cathodic side from the centre of the strip; B, the centre; C, 5 cm to the anodic side from the centre) for checking the movement of a migrant due to the capillary action of the paper, the technique being named the “three-spot method” [6]. Six strips of the support (three pairs of sample solutions and standard solution) were set in parallel in *n*-hexane in the migration chamber and the electrode cells containing 0.1 mol dm⁻³ NaCl solution. A constant electric voltage gradient (1000 V per 30 cm) was applied to the two pairs for 30 min at constant temperature. One pair was allowed to remain in the migration chamber without applying an electric voltage to determine the effect of the capillary action of the support on the movement of a migrant.

BPA on the support was detected by spraying with ninhydrin solution and boric acid with alcoholic curcumin solution.

2.3. IR spectra

Infrared spectra of boric acid in the aqueous carboxylic acids were recorded in the frequency range 1000–2000 cm^{-1} with a Perkin-Elmer Model 1830 Fourier transform IR spectrometer, employing solution cells of 0.025 mm path length with calcium fluoride windows, at ambient temperature (22°C).

3. Results and discussion

3.1. Electrophoretic behaviour of DHBCs

The migration distances (mobilities) of BPA in aqueous solutions of various carboxylic acids are shown in Fig. 1, where a positive distance denotes movement towards the anode and a negative distance towards the cathode. The pH of the supporting solutions was calculated from the $\text{p}K_a$ and the concentration of the carboxylic acids, and is given in Fig. 1. The $\text{p}K_a$ values [17,18] and the chemical forms of the carboxylic acids are summarized in Table 1.

In paper electrophoresis, the observed mobility suffers from the influence of capillary action and the electroosmotic flow caused by the filter-paper as a support. The electroosmotic flow arises from the dissociation of the carboxylic acids in the filter-paper [19]. Hence the effect of the electroosmotic flow is unavoidable in the solutions of $\text{pH} > 2.5$. The influence of the capillary action was checked by the three-spot method. Normally it is minimized by spotting the sample solution in the middle of the filter-paper.

The mobility of a migrant primarily depends on its net charge (Z), which is a function of the $\text{p}K_a$ of the migrant and the pH of the supporting solution. In previous studies [1,2], we showed that the relationship between Z and pH can be expressed to a good approximation by a hyperbolic tangent function:

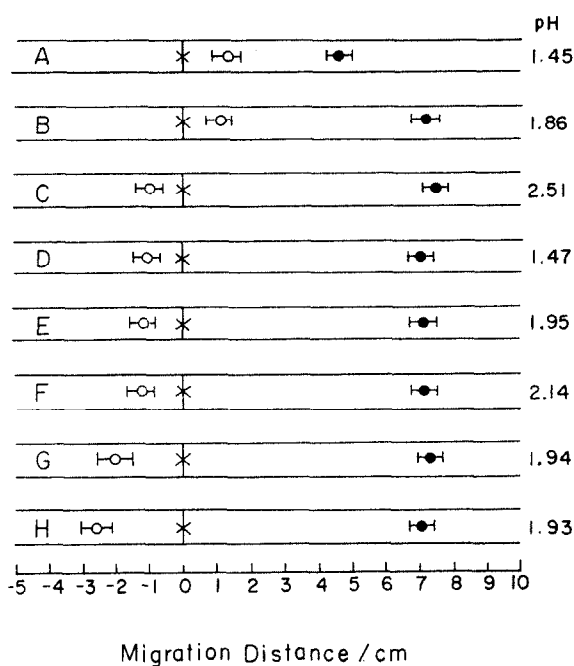
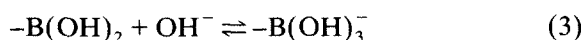
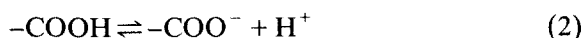


Fig. 1. Observed migration distances of BPA in carboxylic acids: Migrants: ● = picric acid (PA); ○ = BPA. Electrophoretic conditions: 1000 V per 30 cm; time, 30 min; migration temperature, 25°C; support, Toyoroshi No. 51A; spotting position, centre of the support (×); supporting solutions, 0.1 mol dm^{-3} carboxylic acids (lettered as in Table 1).

$$Z = -\frac{1}{2} \sum_{i=1}^n \left\{ 1 + \tanh \left[\frac{2.303}{2} (\text{pH} - \text{p}K_{a_i}) \right] \right\} \quad (1)$$

where K_{a_i} is one of the consecutive dissociation constants of an acid.

The $\text{p}K_a$ values of BPA are 2.46, 8.46 and 9.76 [18] for the dissociation reactions of



The net charge of BPA in a solution of a certain pH was calculated using Eq. 1 and plotted against pH (Fig. 2). In acidic solutions of $\text{pH} < 3$, BPA has a positive charge owing to the NH_3^+ group. In neutral solutions ($\text{pH} 4\text{--}7.5$) BPA has no charge as an amphoteric ion. With increasing pH the negative charge of BPA in-

Table 1
Structural formulae and pK_a values of carboxylic acids, BPA and boric acid

Name	Structural formula	pK_a
(A) Oxalic acid	HOOC-COOH	1.04; 3.82
(B) Citric acid	$\begin{array}{c} \text{CH}_2-\text{C}(\text{OH})-\text{CH}_2 \\ \quad \quad \\ \text{HOOC} \quad \text{COOH} \quad \text{COOH} \end{array}$	2.87; 4.35; 5.69
(C) Fumaric acid	<i>trans</i> -HOOC-HC=CH-COOH	2.85; 4.10
(D) Malic acid	HOOC-CH-CH ₂ -COOH	3.24; 4.71
(E) Tartaric acid	$\begin{array}{c} \text{OH} \\ \\ \text{HOOC}-\text{CH}-\text{CH}-\text{COOH} \\ \quad \\ \text{OH} \quad \text{OH} \end{array}$	2.82; 3.95
(F) Succinic acid	HOOC-CH ₂ -CH ₂ -COOH	4.00; 5.24
(G) Maleic acid	<i>cis</i> -HOOC-CH=CH-COOH	1.75; 5.83
(H) Malonic acid	HOOC-CH ₂ -COOH	2.65; 5.28
BPA	$\begin{array}{c} p\text{-(HO)}_2\text{B-C}_6\text{H}_4\text{-CH}_2\text{-CH(NH}_2\text{)} \\ \\ \text{COOH} \end{array}$	2.46; 8.46; 9.76
Boric acid	B(OH) ₃	9.24

increases according to the reactions 3 and 4. In solutions of $\text{pH} > 11$, BPA has an approximately divalent negative charge.

The closed circles in Fig. 2 show the observed migration distances of BPA at pH 4.0, 6.88 and

9.18 [8]. As expected from the net charge-pH curve, BPA showed negative movement at pH 2. Although BPA has approximately no charge in solutions of pH 4-7, it showed negative movement at pH 4.0 and 6.88. The movement was

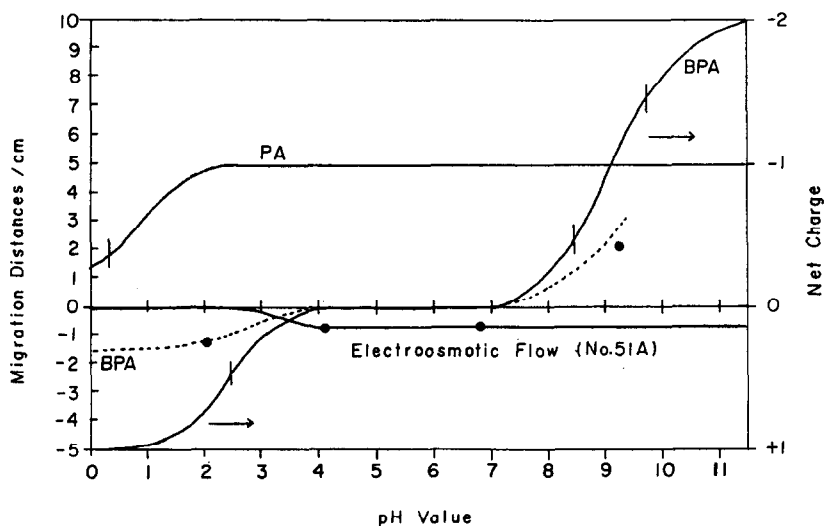


Fig. 2. Net charge and the corrected migration distances of migrants (BPA, PA) at 15°C plotted against pH. The movement due to an electroosmotic flow is shown. Small vertical lines, pK_a ; ● = observed migration distances of BPA at pH 2.0, 4.0, 6.88 and 9.18 [8]; dashed line, migration curve of BPA.

caused by the electroosmotic flow. Based on the observed distances, the electroosmotic flow–pH curve for BPA was obtained. The dashed line in Fig. 2 is the corrected migration curve of BPA for the electroosmotic flow.

The migration distances of BPA in the aqueous carboxylic acids are plotted against pH in Fig. 3. The distances deviate substantially from the migration curve in aqueous oxalic (A) and citric (B) acids, where BPA behaves as an anion. Both oxalic and citric acid are typical ligands [20], and hence it is likely that BPA forms complexes of negative charge with oxalic and citric acids.

In aqueous succinic (F), maleic (G) and malonic (H) acid, the migration distances are in good agreement with those calculated from the migration curve. This indicates no complexation between BPA and each of the three acids.

In aqueous fumaric (C), malic (D) and tartaric (E) acid, BPA behaves as a cation. However, the migration distances deviate somewhat from the migration curve.

Based on the electrophoretic behaviour, the carboxylic acids can be classified into three groups: (i) acids forming complexes of negative charge with BPA (oxalic and citric acids); (ii)

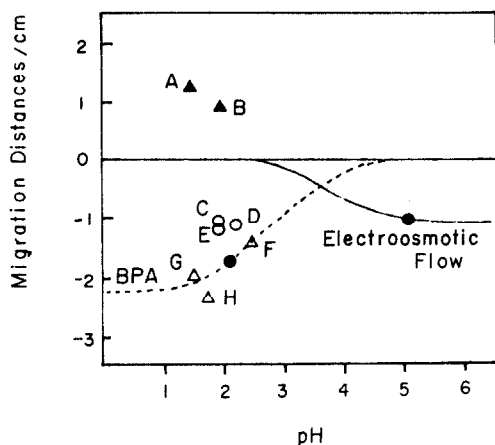


Fig. 3. Observed migration distances of BPA in aqueous 0.1 mol dm⁻³ oxalic acid, citric acid, fumaric acid, malic acid, tartaric acid, succinic acid, maleic acid and malonic acid (lettered as in Table 1). Dashed line, migration curve of BPA.

acids forming complexes of positive charge with BPA (fumaric, malic and tartaric acid); and (iii) acids not forming complexes with BPA (succinic, maleic and malonic acid).

3.2. IR spectra of DHBCs in aqueous carboxylic acids

In order to obtain further evidence for the complex formation, we tried to elucidate the chemical form of the complex by IR spectrometry. As mentioned in the Introduction, orthoboric acid was used as a candidate of DHBCs owing to its moderate solubility in water. Similarly to BPA, orthoboric acid behaved as a cation in oxalic and citric acid but not in maleic acid [21].

Fig. 4 shows the IR spectra of boric acid in water and in aqueous NaHCO₃–Na₂CO₃ solution (pH ≈ 8.5) in the frequency range 1000–

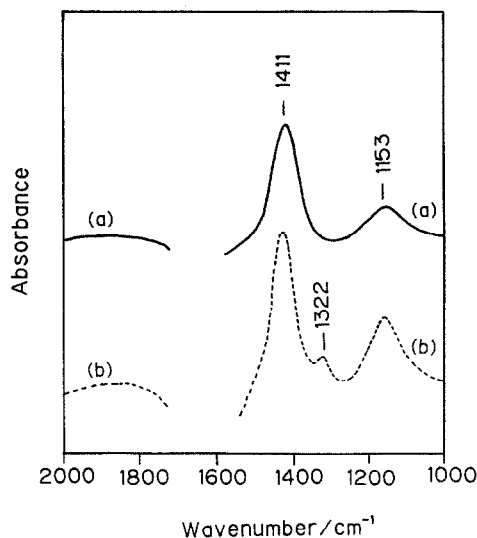


Fig. 4. IR spectra of boric acid in (a) water and (b) aqueous solution of NaHCO₃–Na₂CO₃ (pH ≈ 8.5). Concentration of boric acid, 0.1 mol dm⁻³. Reference: (a) water; (b) aqueous solution of NaHCO₃–Na₂CO₃. Band assignment: 1411 cm⁻¹ = antisymmetric B–O stretching for H₃BO₃; 1151 cm⁻¹ = antisymmetric B–O–H bending for H₃BO₃; 1322 cm⁻¹ = antisymmetric B–O stretching for B(OH)₄⁻. The strong absorption due to the normal water prohibited the spectral measurement in the frequency range 1575–1725 cm⁻¹.

2000 cm^{-1} . The bands at 1411, 1151 and 1322 cm^{-1} are assigned to the antisymmetric B–O stretching for H_3BO_3 , the antisymmetric B–O–H bending for H_3BO_3 [22] and the antisymmetric B–O stretching for $\text{B}(\text{OH})_4^-$ [23], respectively.

The IR spectra of H_3BO_3 in aqueous 0.1 mol dm^{-3} malonic, succinic and maleic acid are shown in Fig. 5. No spectral change was observed in the three acids, indicating that interaction between the dihydroxyboryl compound and each of the three acids was negligible. This coincided with the result of electrophoresis for BPA, namely that the migration distance of BPA towards the cathode approximately agreed with that expected from the charge of the three solutions.

Fig. 6 shows the IR spectra of H_3BO_3 in aqueous oxalic acid. The ratio of oxalic acid molecules to boron atoms (CA/B ratio) ranges from 0.33 to 8.

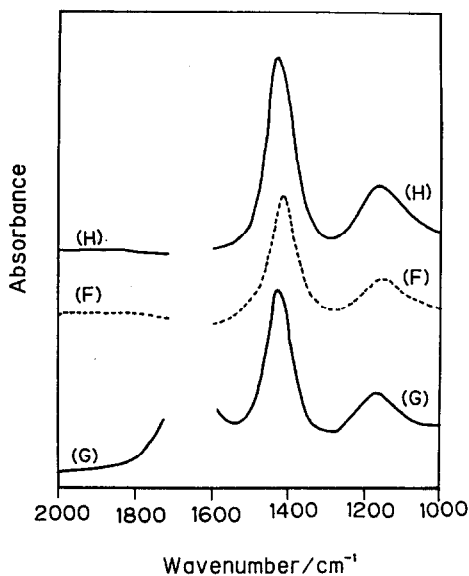


Fig. 5. IR spectra of boric acid in aqueous 0.1 mol dm^{-3} (H) malonic acid, (F) succinic acid and (G) maleic acid. Concentration of boric acid, 0.1 mol dm^{-3} ; ratio of carboxylic acid molecules to boron atoms (CA/B) = 1; reference, aqueous solution of the corresponding carboxylic acid.

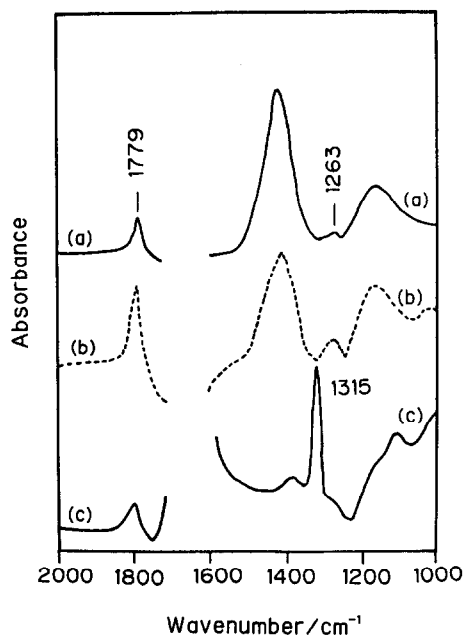
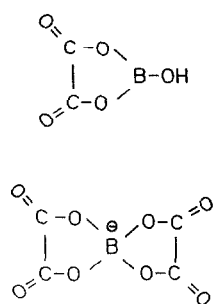


Fig. 6. IR spectra of boric acid in aqueous (a) 0.1, (b) 0.2 and (c) 0.2 mol dm^{-3} oxalic acid. Ratio of carboxylic acid molecules to boron atoms (CA/B): (a) 0.33; (b) 4.0; (c) 8.0. Reference: aqueous (a) 0.1, (b) 0.2 and (c) 0.2 mol dm^{-3} oxalic acid.

The spectrum of H_3BO_3 in a solution with CA/B = 0.33 showed new bands at 1779 and 1263 cm^{-1} , the assignment of which is currently under investigation. In the spectrum for CA/B = 4, the B–O stretching band decreased, whereas the two new bands increased. In the spectrum for CA/B = 8, the two bands due to H_3BO_3 (B–O stretching and B–O–H bending) disappeared almost completely. Instead, the band at 1315 cm^{-1} due to the $\text{B}(\text{OR})_4^-$ group increased.

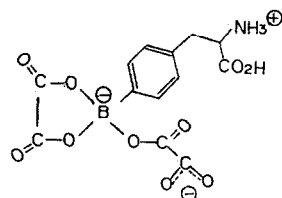
The spectral behaviour can be explained as follows. In solutions of lower CA/B ratio, boric acid and oxalic acid form a complex (complex III), in which the boron atom is trivalent. The complex III bonds to another oxalic acid molecule in the solutions of larger CA/B ratio, giving another complex (complex IV) with tetrahedral boron.

For the complexes of boric acid with oxalic acid, Van Duin et al. [24] proposed the following chemical species:



It is possible to identify the complex **III** with a chemical form comparable to the top formula and the complex **IV** to the bottom formula. Taking the spectral result into account, we explain the electrophoretic behaviour as follows: many oxalate ions are located around the dihydroxyboryl compounds in the background solution in electrophoresis, and hence the boron compounds form complexes such as the complex **IV** with negative charge.

Based on the spectral behaviour of boric acid and the migration distance of BPA in aqueous citric acid, we propose the following chemical form for the complex of BPA with oxalic acid:



In aqueous citric acid, similar spectral changes were observed with increasing CA/B ratio, as shown in Fig. 7. Clearly, boric acid formed a tetrahedral boron complex with citric acid.

Fig. 8 shows that boric acid forms complexes with fumaric, malic and tartaric acid. However, no evidence was detected for tetrahedral boron complexes.

In conclusion, complex formation of dihydroxyboryl compounds with oxalic and citric acid

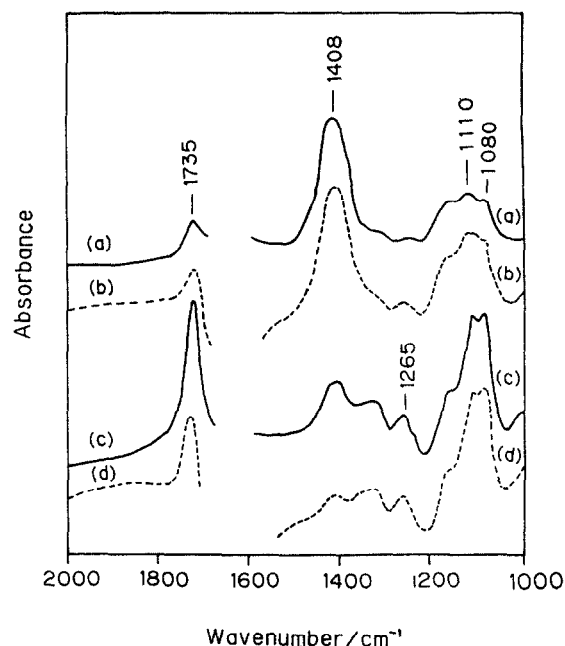


Fig. 7. IR spectra of boric acid in aqueous (a) 0.1, (b) 0.1, (c) 0.2 and (d) 0.2 mol dm⁻³ citric acid. Concentration of boric acid: (a) 0.2; (b) 0.1; (c) 0.05; (d) 0.025 mol dm⁻³. Ratio of carboxylic acid molecules to boron atoms (CA/B): (a) 0.5, (b) 1.0; (c) 4.0, (d) 8.0.

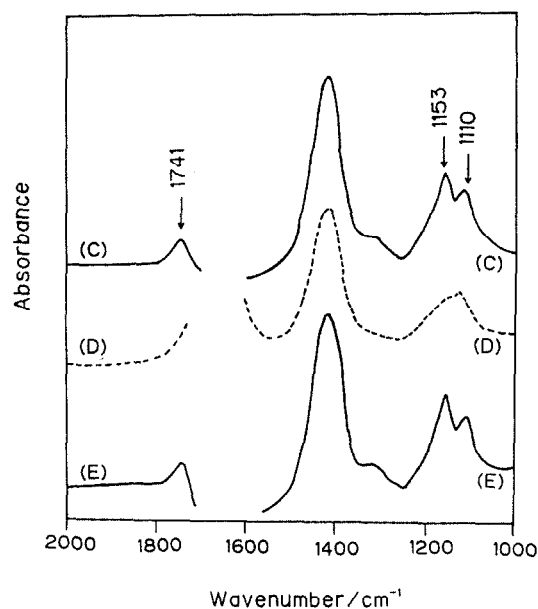


Fig. 8. IR spectra of boric acid in aqueous 0.1 mol dm⁻³ (C) fumaric acid, (D) malic acid and (E) tartaric acid. Concentration of boric acid: (C) 0.13; (D) 0.1; (E) 0.13 mol dm⁻³. Ratio of carboxylic acid molecules to boron atoms (CA/B): (C) 0.75; (D) 1.0; (E) 0.75.

and also with fumaric, malic and tartaric acid was predicted by paper electrophoresis and was proved by IR spectrometry. No appreciable interaction was detected between boric acid and succinic, maleic or malonic acid. The spectral behaviour was fully consistent with the migration behaviour in paper electrophoresis. It was confirmed that electrophoresis is useful not only for separating substances but also for elucidating the chemical states of a compound in solution.

4. References

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