Reversed-Phase High-Performance Liquid-Chromatographic Behavior of Phthalic Acid and Terephthalic Acid in the pH Region around the Second pK_a Values

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Synopsis. In a reversed-phase high-performance liquid-chromatographic analysis phthalic acid and terephthalic acid were observed as two clearly separated peaks with a mobile phase of pHs around the second pK_a values involving the monoanion and dianion species.

A reversed-phase high-performance liquid-chromatographic (HPLC) method is widely applied for the analysis of biological substances and chemicals involving polar groups, such as carboxyl, carbonyl, hydroxyl, and amino groups.[1,2] For ionic compounds, the pH of a mobile phase is controlled so as to avoid the generation of ionic species (an ion-suppression method) in order to obtain good HPLC separation.[3] Ionic species produced by dissociation, however, are present in many ion-suppression HPLC systems for the separation of strong acids and bases, being separated by an aliphatic difference in the moiety except for dissociation groups. Such secondary equilibrium in a solution is sometimes used to control the retention in order to obtain good chromatograms. We have come across an observation of two separated peaks for phthalic acid and terephthalic acid using a mobile phase in the pH region at around the second pK_a (pK_{a2}) values. In this paper we consider what conditions caused the detected peak of phthalic acid and terephthalic acid to separate into two, in order to extend the reversed-phase HPLC analysis technique for ionic species.

Experimental

HPLC System. A Shimadzu HPLC system was used; it comprises two HPLC pumps (LC-9A) connected to a high-pressure GE mixer, an on-line degasser (DGU-4A), a photodiode array UV-vis detector (SPD-M6A) with an NEC personal computer (PC-9801RA2) for data analyses, an UV-vis spectrophotometric detector (SPD-6AV) set at 290 nm with a chromatopack (CR-4A), a column oven (CTO-6A) regulated at 25 °C, a system controller (SCL-6B), and an auto injector (SIL-6B). The HPLC separations were usually performed on a Chromatorex C_{18}-5 column (Fuji-Davison Chemical Ltd.) of 4.6 mm i.d.×250 mm, and a Capselpack C_{18}-5 column (Shiseido) of 4.6 mm i.d.×250 mm at a flow-rate of 1.0 ml min^{-1}. A Chromatorex C_{18}-5 column (Fuji-Davison Chemical Ltd.) of 4.6 mm i.d.×150 mm was used to clarify the effect of the column length on the chromatography.

Chemicals and Solvents Used as a Mobile Phase. Phthalic acid, terephthalic acid, and benzoic acid were commercially available from Nacalai Tesque, Inc., and were recrystallized twice from ethanol before use. All other reagents were of the highest purity available. Mobile phases consisting of 0.2 mol dm^{-3} acetate, malonate, tertric, or phosphate buffer including CH_3CN were used to examine the pH effect on the separation behavior. The ionic strength of the buffer solutions was adjusted to 0.6 with NaCl. In this paper the pH values of a mobile phase are described as the pH values of the buffer solutions before adding CH_3CN, since it is difficult to measure the true pH values of an aqueous solution containing CH_3CN with the usual glass electrode.[4] In the case of preparing sample solutions, a 0.05 mol dm^{-3} NaOH stock solution of the sample was first prepared; it was then usually diluted 10 times with the same buffer solution as the mobile phase used for HPLC separations, the final concentration being 9.50×10^{-4} mol dm^{-3}. A 10-μl aliquot of the sample solution was injected into an HPLC column.

Results and Discussion

Observation of Two Separated Peaks for Phthalic Acid and Terephthalic Acid in Reversed-Phase HPLC Analysis. Phthalic acid as well as terephthalic acid is a dicarboxylic acid, as shown in Fig. 1. The monoanion and dianion produced by dissociation are present in the second equilibrium. The use of a mobile phase containing a buffer solution having a pH of around the pK_{a2} value gave two separated peaks on the chromatograms of phthalic acid, as can be seen from Fig. 2. This behavior is reproducibly observed, and is sensitively affected by the ratio of CH_3CN in the mobile phase, as shown in Fig. 3. Since the addition of CH_3CN to a buffer solution suppress the acid-dissociation of the buffer component, the proton concentration decreases. In fact, the two clearly separated peaks were observed at various concentrations of CH_3CN in the mobile phase, being isometrically recorded at pH 5.40, 5.35, 5.25, 5.10, and 4.90 for CH_3CN concentrations of 0, 5, 10, 15, and 20%, respectively. Since the pH value of the mobile phases deviates from that of the buffer solution in the acidic direction, the split peaks seem to be observed at a true pH close to the pK_{a2} value. The above-mentioned behaviors were also observed in the HPLC separation of terephthalic acid by the use of a

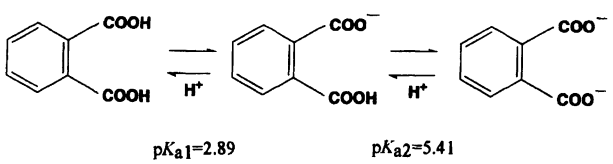

Fig. 1. Acid-dissociation equilibrium and the pK_a values of phthalic acid.

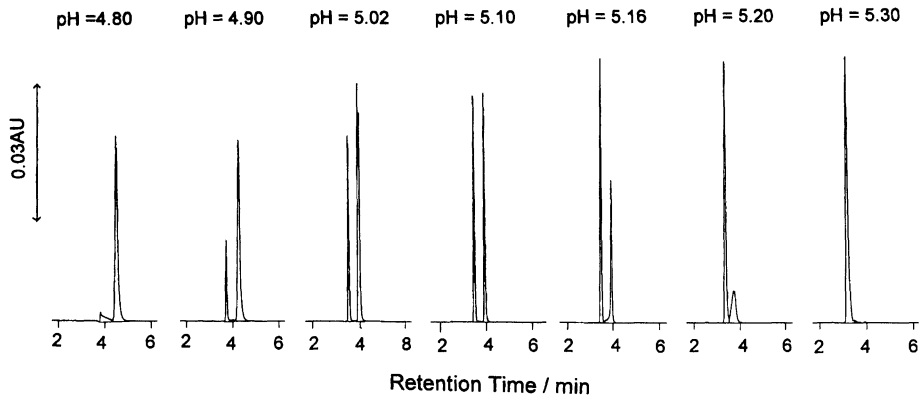

Fig. 2. Change in chromatograms of phthalic acid with pH of the mobile phases. Mobile phases consist of 0.2 mol dm^{-3} acetate buffer, pH being assigned in the figure, and CH_3CN (15%). The sample preparation and the HPLC conditions are described in the experimental section.

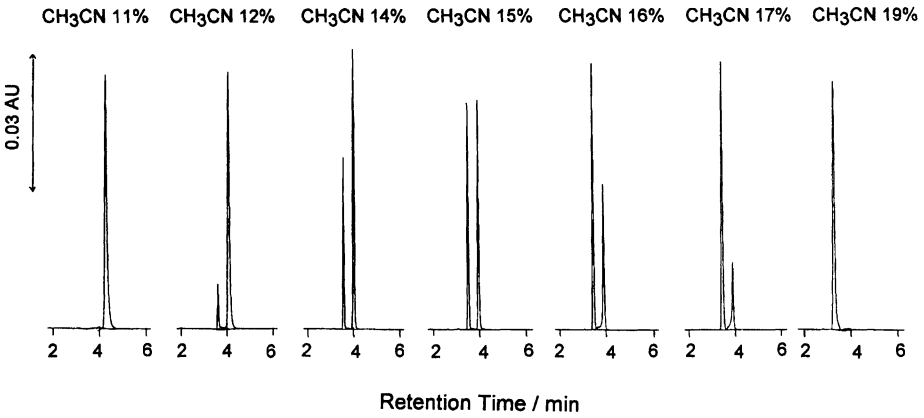

Fig. 3. Change in chromatograms of phthalic acid with the concentration of CH_3CN. Mobile phases consist of 0.2 mol dm^{-3} acetate buffer (pH 5.10) and CH_3CN of which the concentration are assigned in the figure. The sample preparation and the HPLC conditions are described in the experimental section.

mobile phase of pH around the pK_{a2} value. Note here that this kind of behavior does not happen to be observed with a specific apparatus, but can be observed with all the HPLC systems and all the C_{18} columns as far as we can use. The use of unmodified and C_5 modified silica-gel columns never caused the peak of phthalic acid to split.

A three-dimensional chromatogram for phthalic acid is given in Fig. 4, corresponding to the simple chromatogram at pH 5.10 in Fig. 2. It is found that the detection of the two peaks arises in the same spectra. The spectra shown in Fig. 4 are the same as those obtained by spectral measurements for phthalic acid in a buffer solution of pH 5.40 (=pK_{a2}). The two peaks detected isometrically in Fig. 2 (pH 5.10) therefore correspond not to the monoanion and dianion species of phthalic acid respectively, but to a 1 : 1 mixture of those. This indicates that the true proton activity in the mobile phase containing CH_3CN, in which the isometrical two peaks are observable, is equal to the pK_{a2} value. It would be very interesting to know the true pH value in an aqueous–organic medium.

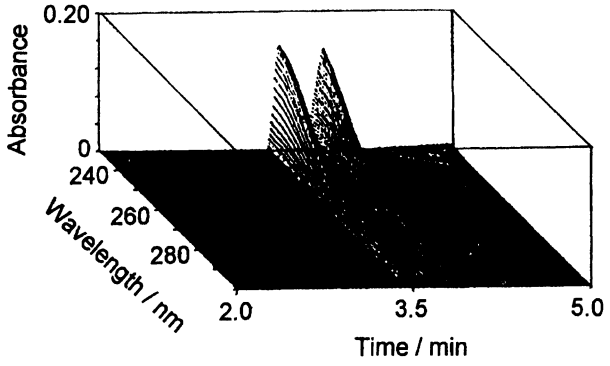

Fig. 4. Three dimensional chromatogram for phthalic acid involving retention time, absorbance and wavelength axes. A mobile phase consists of 0.2 mol dm^{-3} acetate buffer (pH 5.10) and CH_3CN (15%).

Relation between the Retention Time and the pH of the Mobile Phases. Figure 5 shows the relation between the retention time and the pH on chromatograms of phthalic acid and benzoic acid. Varying the pH of a mobile phase from 3 to 6 greatly decreased

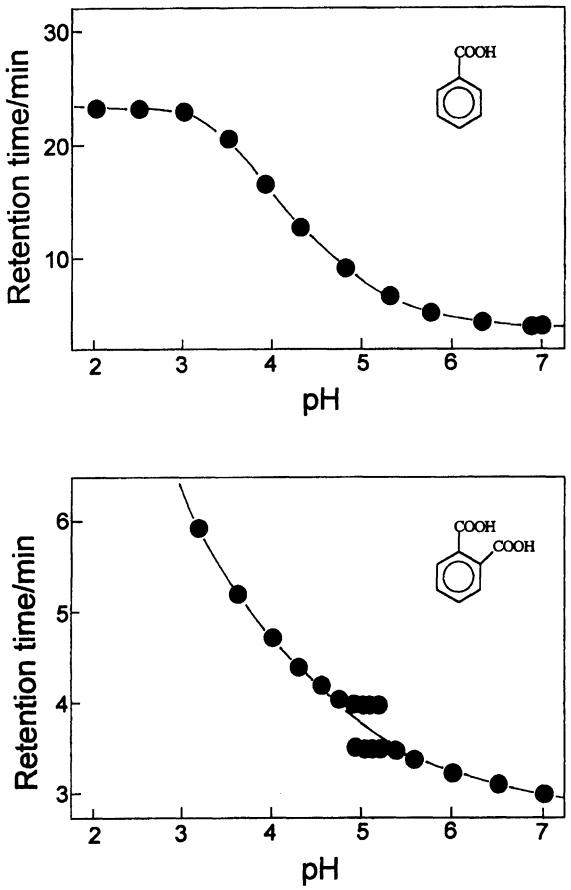

Fig. 5. Relation of the retention time and pH in chromatogram of benzoic acid and phthalic acid. Smooth curves join the experimentally obtained points. For the pH region in which the two peaks were observed for phthalic acid the curve represents the gravity mean of the two peaks. See the text for details.

the retention of benzoic acid (pK_a=4.20), corresponding to a decrease of the existence ratio of neutral to anion species, since the acid is considered to be quite undissociated and fully dissociated to an anion in a lower pH region than 2.5 and a higher pH region than 6.5, respectively. This is a well-known behavior for partition-dominated HPLC separation involving secondary acid-dissociation equilibrium. The elution time of phthalic acid also decreases with increasing the basicity of the mobile phase in pH regions lower than 4.8 and higher than 5.3. In the 4.8—5.3 pH region the peak on the chromatogram of phthalic acid splits in two, and the retention time of the two peaks is independent of the pH of a mobile phase, keeping the peak separation almost constant (about 0.45 min), as can be seen from Fig. 5. The curve for phthalic acid in Fig. 5 was drawn in the 4.8—5.3 pH region as follows. The gravity means (T_{mean}) of the two peaks involving the time and the peak height were calculated using

$$T_{mean} = \frac{t_1 h_1 + t_2 h_2}{h_1 + h_2},$$

where t_1 and t_2 are the retention time for the two peaks, and h_1 and h_2 are the peak heights of those. The T_{mean} values were plotted against the pH values, and joined by a smooth curve. This curve was smoothly joined by a curve representing the relation between the retention time and the pH value.

The use of a column of 15-cm length as well as that of 25-cm length allowed the peak of phthalic acid and terephthalic acid to split in two by the mobile phases of the pH around the pK_{a2} values. In the case of the 15-cm column, the retention time shifted in the direction of shorter time compared to the case of the 25-cm column, keeping the time difference of the two separated peaks at about 0.45 min. This seems to tell us that the split in the peak is caused at the very beginning of the separation. Although both of the peak heights linearly depend on sample concentrations, an increase in the injection volume makes the relative height of the two peaks changed. When the samples were dissolved in the same solution as the mobile phase used in HPLC separation, the two clearly separated peaks merged into a single peak with two points. It therefore seems that the partition equilibrium of ionic species between the mobile and stationary phases is disturbed at the inlet of the column[5] when monoanion and dianion species exist in almost equal quantities. Thus, the observation of the two clearly separated peaks has been considered to be a particular phenomenon in a reversed-phase HPLC analysis with C_{18} columns involving secondary equilibrium between the dissociated monoanion and dianion species. In fact, the addition of tetrapropylammonium bromide to the mobile phase allows the two separated peaks of phthalic acid to become one sharp peak.

In conclusion, two separated peaks were observed in the presence of the monoanion and dianion species in almost equal quantities; the behaviors are sensitively affected by the percentage of organic solvents in the mobile phase. It seems that the two peaks appear due to a disturbance of the partition equilibrium at the very beginning of the separation. The separation mechanisms in the present system should be elucidated more clearly in future work in order to explore a wider applicability, and not to erroneously identify a peak in a reversed-phase HPLC system for ionic species.

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