Behavior of Weak Acid Anions in Response to Changes in Mobile Phase pH in Indirect Photometric Chromatography

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Abstract

Indirect photometric chromatography involves the separation of analytes via ion-exchange chromatography and indirect photometric detection. Weak organic acids are typically used as eluting ions. Changes in mobile phase pH affect the speciation of the analytes and thus affect the retention and detection characteristics of analytes. The situation is complicated in the case of weak acid eluents whose speciation is also affected by the pH changes. In this study, the retention and detection characteristics of several weak and strong acids were studied as a function of mobile phase pH with a phthalate-based eluent. Trends in both the detection and retention properties of the weak acids reflected the changing speciation and provided estimates of the dissociation constants of the acids.

Introduction

Indirect photometric chromatography (IPC), which has been described by Small and Miller (1), couples analyte separation by ion-exchange with indirect photometric detection. Indirect detection results from the reduction in the background absorbance of the mobile phase that occurs when a transparent analyte displaces an ultraviolet-active mobile phase component (e.g., phthalate) during elution. Since the detector response is related to the background absorbance of the mobile phase, IPC can enhance sensitivity for analytes that are otherwise difficult to detect and has been applied to pharmaceutical analysis (2,3). In addition, because the magnitude of the detector response per equivalent of a transparent analyte is independent of analyte identity, several authors have observed that IPC is amenable to "standardless" calibration (4,5). In other words, the response factor (detector signal per equivalent of analyte) for all analytes in a given mobile phase is the same, and thus, the response factor obtained with any analyte is readily applicable to other analytes. Therefore, an unidentified analyte can be quantitated by using the response factor obtained from a known analyte. If the charge of the unidentified analyte is known, then its molar concentration can be calculated.

Changing the pH of the mobile phase has a profound effect on the nature of an IPC separation because IPC uses a weak acid as an eluent. As the mobile phase pH changes, the eluting power and ultraviolet absorption spectrum of the eluent changes. The impact of these mobile phase changes on the retention and detection of strong acid analytes can readily be determined (6–8) because the charge of the analyte is not affected by changes in the mobile phase. However, since the speciation (and thus effective charge) of a weak acid analyte is also affected by mobile phase pH, the effect of changes in the mobile phase pH on the retention and detection characteristics of weak acids is more complex. Although several authors have examined the effect of mobile phase pH on the retention characteristics of weak acid analytes in ion chromatography (9,10), few IPC studies have considered the effect of mobile phase pH on detector response.

The purpose of this research was to examine the effect of changes in the mobile phase pH on the elution and detection properties of both weak and strong acid analytes in IPC. It was anticipated that the behavior of the weak acids would provide some indication of their dissociation constants.

Experimental

Apparatus

The chromatographic system consisted of a Waters Model 510 pump (Bedford, MA), an electronically actuated Rheodyne 7010 injector (Cotati, CA), an Alcott Model 728 autosampler (Norcross, GA), a Waters IC-PAK anion column, a Kratos Model 757 ultraviolet detector (Ramsey, NJ), a strip chart recorder, and a Hewlett-Packard 3357 LAS computer data system (Palo Alto, CA). Ultraviolet spectra were obtained by using a Hewlett-Packard HP8452A photodiode array spectrophotometer.

Procedure

Mobile phases were prepared to contain 0.5mM potassium hydrogen phthalate and were adjusted to pH values of 4.0, 4.5, 5.0, 5.5, 6.0, and 6.6 with 0.1N sodium hydroxide. The mobile phases were filtered through 0.45-µm filters. Analytes that were examined included chloride, nitrate, sulfate, phosphate, malonate, acetate, and formate. Single analyte standards were pre-

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pared at a concentration of approximately 0.2 meq/L by diluting concentrated stock solutions in each mobile phase examined. In this way, the sample was equilibrated with the mobile phase (especially with respect to pH) before injection.

The ultraviolet absorbance of each mobile phase was measured over the wavelength range of 220–280 nm by using 1-cm quartz cells and water as the reference. The chromatographic separations were obtained with a mobile phase flow rate of 1.2 mL/min, an injection size of 50 µL, and detection wavelengths of 260 and 280 nm for each mobile phase. The mobile phases used were changed in a random fashion, and sufficient time was allowed after a change for the system to equilibrate. Each analyte was individually injected in triplicate for each mobile phase and detection wavelength set.

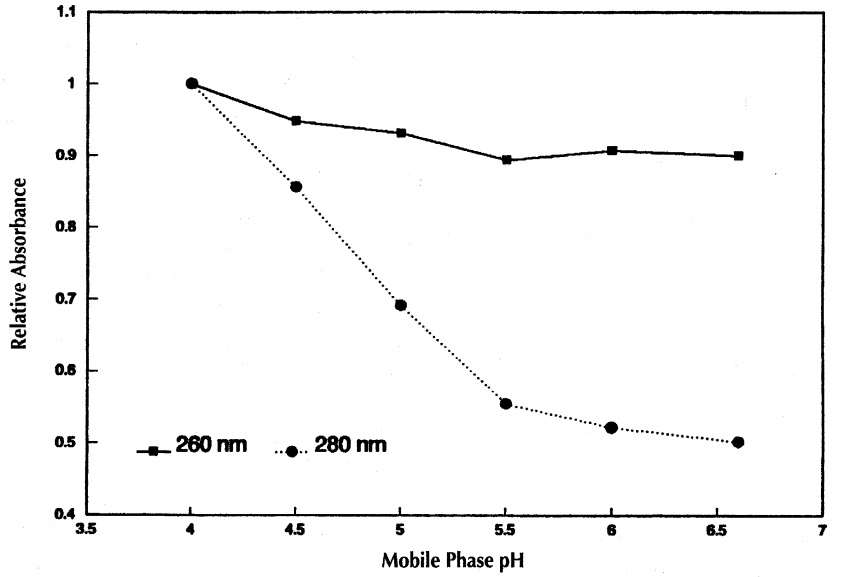

Figure 1. The effect of mobile phase pH on the absorbance of the eluent ion (phthalate). The divalent ions, which dominate at higher pH, have a lower molar absorptivity than the monovalent forms present in excess at low pH. As the pH increases, the magnitude of both the background absorbance of the eluent and the response of the analyte decreases. The magnitude of the decrease is dependant on the wavelength and is minimized at the isobestic point (262 nm).

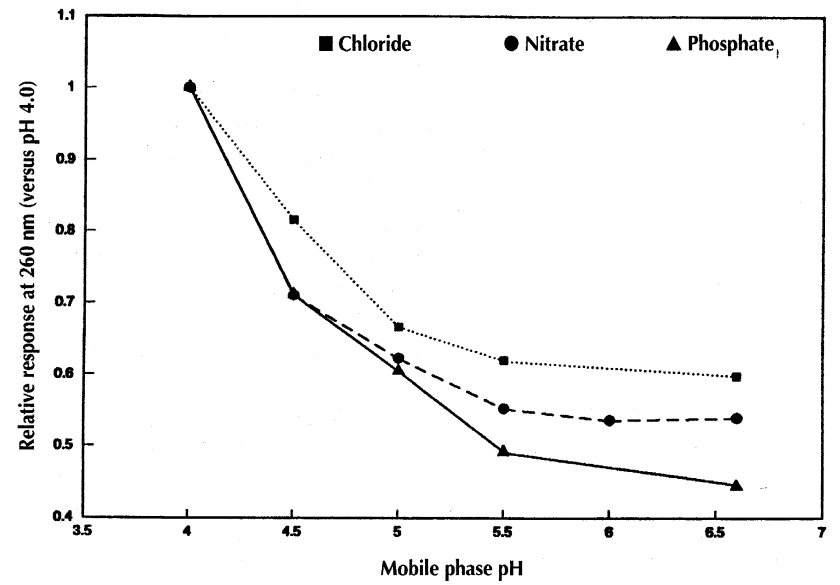

Figure 2. Effect of mobile phase pH on detector response (at 260 nm) of several strong acids. The detector response is expressed as the fraction of the response observed at a mobile phase pH of 4.0. As the pH increases and the equivalent absorptivity of the eluent decreases, the response of the analyte decreases in a manner described by a smooth curve.

Results and Discussion

The analytes examined included strong acids (chloride, nitrate, and sulfate) and both monoprotic (acetate and formate) and multiprotic (malonate and phosphate) weak acids. Acid dissociation constants for the weak acids (obtained from reference 11) included the following: acetate, 4.75; formate, 3.75; malonate, 2.80, 6.10; phosphate, 2.00, 7.00, 12.00; and phthalate, 3.10, 5.40.

Both the weak acid analytes and the eluent underwent significant changes in speciation over the pH range studied. In the case of the eluent, changed phthalate speciation from one dominated by the monovalent species at the lowest pH examined (pH 4.0) to one dominated by the divalent species at the highest pH examined (pH 6.6). This change in speciation can be quantitatively expressed in terms of the effective charge of the ion (e), where e is the sum, for all forms of the ion, of the charge of the form (c) and the fraction of the species that is in that form (f). For a divalent analyte, e is calculated as

$$e = 1(f_1) + 2(f_2)$$

where f_1 and f_2 are the fractions of the species in its monvalent (charge of 1) or divalent (charge of 2) form. The magnitudes of f_1 and f_2 are controlled by the pH of the solution and by the pK_a value(s) of the analyte. The resulting near unit change in effective charge of the phthalate eluent (0.93 at pH 4.0 and 1.94 at pH 6.6) affects both the retention and the detection of analytes in general. In the case of retention, the increase in the effective charge of the eluent resulted in a stronger mobile phase and reduced retention. In terms of detection, the increased effective charge meant that although the same number of equivalents of the eluent were displaced by an analyte at both pH extremes, fewer moles of eluent were displaced at higher pH values. Thus, in absolute terms, the analyte signal was smaller at higher pH values. This effect was further exacerbated by the ultraviolet absorption characteristics of the monovalent

and divalent forms of the phthalate ion. As shown in Figure 1, the molar absorptivity of the divalent species was smaller than that of the monovalent species. The magnitude of the difference in molar absorptivity was dependent on the wavelength; the smallest difference occurred at wavelengths near the isobestic point of phthalate (previously identified as 262 nm in reference 12). Again, the effect of this behavior on detection was that analyte signal was reduced as the pH of the mobile phase increased.

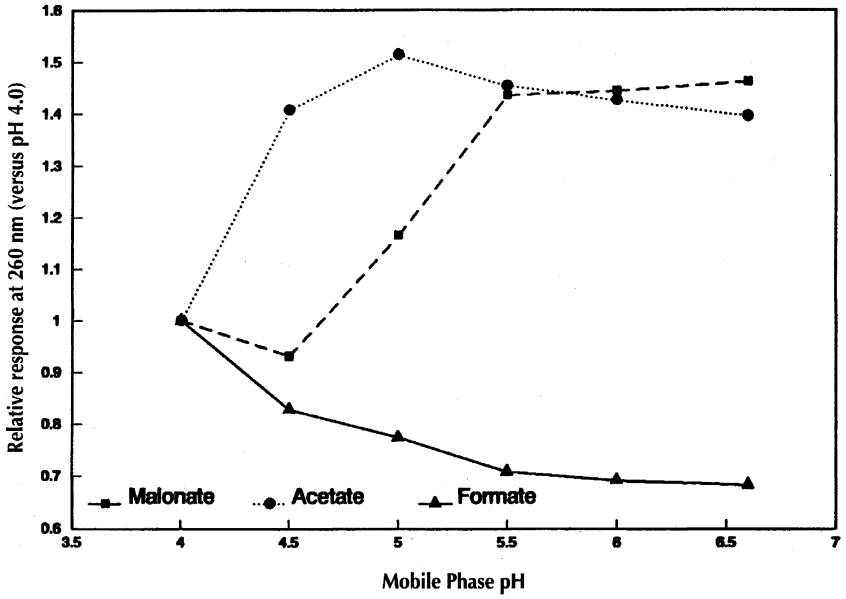

Figure 3. Effect of mobile phase pH on the detector response (at 260 nm) of several weak acids. The detector response is expressed as the fraction of the response observed at a mobile phase pH of 4.0. The increased effective charge of the acids near their pK_a values offsets the decreased equivalent absorptivity of the eluent with the net result that the response of the analyte increases in the region near the their pK_a values decreases in a manner described by a smooth curve.

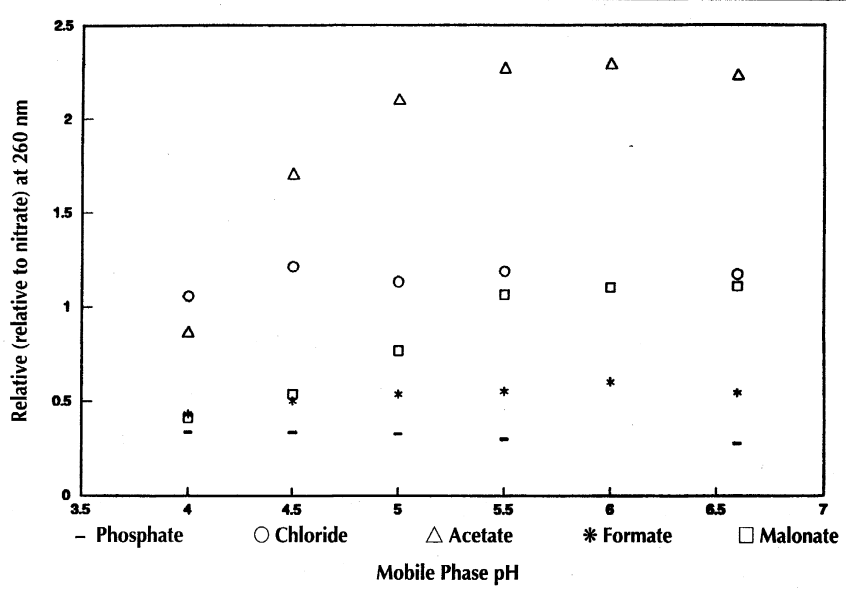

Figure 4. Effect of mobile phase pH on the relative detector response of the analytes examined. The relative detector response is the ratio of the detector response for a particular analyte divided by the detector response for nitrate. The detection wavelength used was 260 nm. The relative response ratio of strong acids is constant because the strong acids exhibit one charge. However, the response ratios for the weak acids increase as the pH approaches and exceeds the pKa value of the acids because of their increase in effective charge with an increase in pH. Thus the relative response plots can be used to estimate the pKa of a weak acid.

On the basis of this analysis, it can be anticipated that the detector response of analytes will decrease as the mobile phase pH increases. The absolute magnitude of the effect reflects the combination of the lower molar absorptivity of the divalent phthalate ion and the higher effective charge of the phthalate eluent. It was expected that the decrease in analyte response would be dependent on the wavelength because the difference in molar absorptivities is dependent on the wavelength. For strong acid anions, the anticipated behavior was observed, as is shown in Figure 2. For chloride, nitrate, and phosphate, the detector response decreased with increased eluent pH. However, the detector response for the weak acid anions (Figure 3) showed a different response to changes in the mobile phase pH. For formate, which has a pK_a of 3.75, the magnitude of the decrease in detector response was much smaller than that of the strong acid anions. For acetate, which has a pK_a of 4.75, the detector response increased dramatically between pH 4.0 and 5.0 and then began to decrease at a mobile phase pH greater than 5.0. For malonate, which has a second pK_a of 6.10, the detector response increased above pH 5.0. This behavior reflected the change in speciation that the weak acids underwent over the pH range studied. As was the case with phthalate, the speciation of the weak acid analytes shifted in favor of their higher valent form at higher pH values. Thus the effective charge of the weak acid analytes increased with increases in pH. The net result was that the analytes displaced a larger molar quantity of the phthalate ion. This resulted in a greater detector response for the analytes as the mobile phase pH increased. Although phosphate is also a weak acid and has pK_a values of 2.00 and 7.00, its speciation was not strongly impacted in the pH range studied; therefore, it behaved like a strong acid with respect to its detector response.

This phenomena can also be illustrated in terms of changes in relative detector response. In this discussion, the relative response is defined as the ratio of the detector response for a given analyte versus that of a reference analyte. If a strong acid (e.g., nitrate) was chosen as the reference ion, it was expected that the relative response of other strong acids would not change as a

function of mobile phase pH but the response ratio for a weak acid would increase as the mobile phase pH approached the pK_a values of the weak acids. As is shown in Figures 4 (260 nm) and 5 (280 nm), this behavior was observed for the analytes studied.

The effect of changes in mobile phase speciation on the retention of strong and weak acid analytes has been addressed by several authors who have considered both phthalate-based and carbonate-based weak acid eluent systems (4–8). Qualitatively, the retention of strong acid analytes decreased as the effective charge of the eluent ion increased in response to an increased mobile phase pH. As noted previously, the greatest change in effective charge for the phthalate eluent occurred between pH 4 and 6, as the speciation changed from predominantly monovalent to predominantly divalent. Above pH 6, further increases in pH had little effect on phthalate speciation. Thus, it would be expected that the retention of strong acid analytes would decrease significantly as the mobile phase pH increases from 4 to 6 with relatively minor changes occurring above this pH. As shown in Figures 6 and 7, such behavior was exhibited by chloride, nitrate and sulfate. Changing the mobile phase pH had the most profound effect on the retention of sulfate (versus chloride or nitrate) because this analyte is divalent.

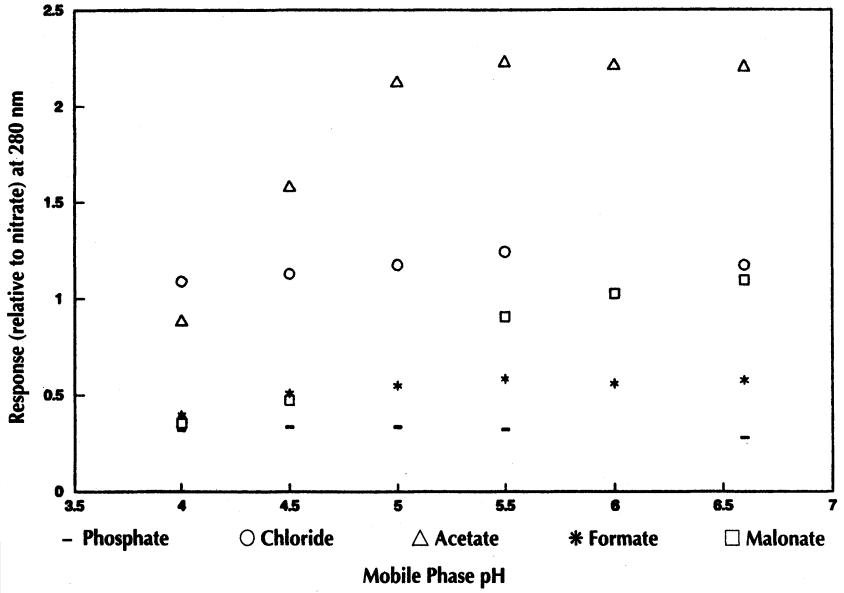

Figure 5. Effect of mobile phase pH on the relative detector response of the analytes examined. The relative detector response is the ratio of the detector response for a particular analyte divided by the detector response for nitrate. A detection wavelength of 280 nm was used. The observations made for Figure 4 are also valid at a second detection wavelength.

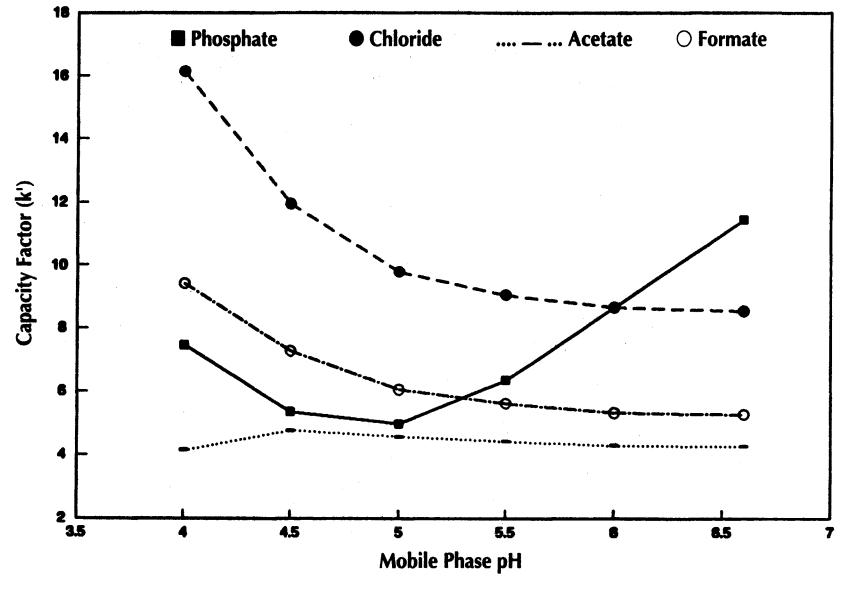

Figure 6. Effect of mobile phase pH on analyte retention (capacity factor). Increased mobile phase pH results in a stronger, higher effective charge for the eluent, which decreases the capacity factor for the strong acids (e.g., chloride). However, since the effective charge of the weak acids (e.g., acetate, phosphate, and formate) also increases with increasing pH, their capacity factors increase near the pK_a values of the acids.

Figures 6 and 7 also show that the effect of changes in mobile phase pH on the retention of the weak organic acids is less orderly. The behavior of the weak acid analytes, which is typified by phosphate, represented the competing effects of pH on the speciation (and effective charge) of both the analyte and eluent ions. As noted previously, although the eluting strength of the mobile phase increases with increased mobile phase pH (thus decreasing retention), this effect is counterbalanced by the increasing effective charge of the analyte, which would tend to result in increased retention. In a pH region in which the effective charge of the analyte ions is relatively constant but the effective charge of the eluent ions increases (pH between 3.8 and 5.5 for the phosphate–phthalate couple), the retention response of the analyte to changes in pH is similar to that of a strong acid and decreased retention was observed. At some pH, the change in effective charge of the analyte will be greater than the change in effective charge of the eluent, and retention will increase with increasing pH. Once the mobile phase pH increases past the pK_a of weak acids, the impact of further changes in pH on the effective charge of the analyte will be diminished. In such cases, retention will either remain constant or decrease slightly in response to further mobile phase pH increases (e.g., acetate above pH 4.5).

The detection and retention characteristics of weak acid analytes showed distinctive behavior in response to changes in the mobile phase pH in the pH region around their pK_a value(s). This behavior, which was shown in plots of retention (or detector re-

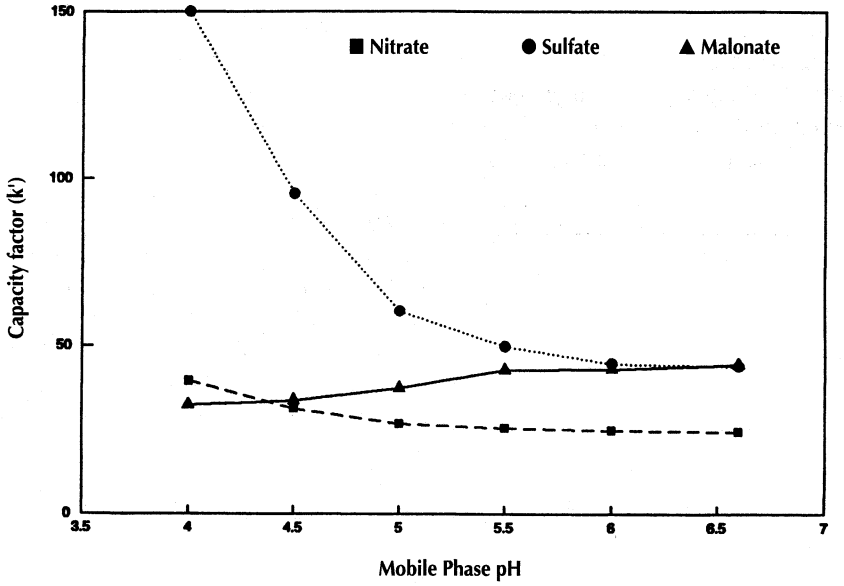

Figure 7. Effect of mobile phase pH on analyte retention (capacity factor). Increased mobile phase pH results in a stronger, higher effective charge for the eluent, which decreases the capacity factor for the strong acids (nitrate and sulfate). However, since the effective charge of the weak acid (malonate) also increases with increasing pH, the capacity factors increase near the pK_a value of the acid. This behavior of the weak acids may be used to estimate their pK_a values.

sponse) versus mobile phase pH, allowed the estimation of pK_a values. For example, one would correctly estimate a pK_a of approximately 6 for malonate on the basis of the shape of the relative detector response (Figure 4) and retention versus pH plots (Figure 7). However, the accuracy of the estimate is limited by the complicated nature of the changes that occur in detector response and retention as the speciation of both the eluent and analyte species changes in response to mobile phase pH changes. It was anticipated that the accuracy of such an estimate would be increased significantly if the speciation of the eluent ion was unaffected by pH. However, it is difficult to identify an eluent that exhibits the desired speciation and possesses the required ultraviolet absorption properties to facilitate the indirect photometric detection.

Conclusion

Observation of the trends in retention and detector response as a function of changing mobile phase pH in indirect photometric chromatography can provide estimates of the dissociation constant of weak acid analytes. These trends result from the changing speciation of these analytes in response to mobile phase pH. The nature of the trends is complicated when weak acid eluents are used because their speciation is also affected by the changing mobile phase pH. In general, however, the detector response for a weak acid analyte will increase as the pH of the mobile phase increases in the region of the pK_a of ther analyte. Thus the concept of "standardless" calibration, wherein the response factor obtained for one primary analyte is used to quantitate secondary analytes, cannot be universally applied in situations in which the identity or nature of the secondary analyte is unknown.

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