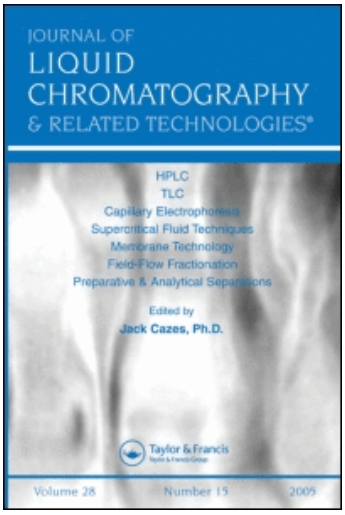

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Changes in Retention and Detector Response for Weak Acid Anions as a Function of Mobile Phase pH in Indirect Photometric Chromatography with a Singly Charged Eluent Ion

D. R. Jenke[a]
[a] Corporate Research and Technical Services Baxter Healthcare Corporation William B. Graham Science Center, Round Lake, IL, USA

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CHANGES IN RETENTION AND DETECTOR RESPONSE FOR WEAK ACID ANIONS AS A FUNCTION OF MOBILE PHASE pH IN INDIRECT PHOTOMETRIC CHROMATOGRAPHY WITH A SINGLY CHARGED ELUENT ION

Dennis R. Jenke

Corporate Research and Technical Services
Baxter Healthcare Corporation
William B. Graham Science Center
Round Lake, IL 60073 USA

ABSTRACT

Indirect photometric chromatography (IPC) involves analyte separation by ion exchange and detection using indirect UV photometry. Since retention and detection are impacted by the speciation of the weak acid analytes and eluents, both are influenced by mobile phase pH. In this study, the retention and detection characteristics of several weak and strong acids were studied as a function of mobile phase pH with an eluent whose speciation was only marginally impacted by pH. In so doing, analyte ion effects are divorced from the eluent ion effects which are typically observed with the weak acid eluents used in IPC. Trends in both the detection and retention characteristics of the weak acids mirrored their changing speciation and could be used to estimate their acid dissociation constants.

INTRODUCTION

Indirect photometric chromatography (IPC) is an ion chromatographic method utilizing aromatic weak acid eluents to achieve both the desired analytical separation and detection.[1,2] As is the case with most classical IC procedures, separation is accomplished with low capacity weak acid ion exchange resins and a mobile phase which contains one or more eluting ions. However, IPC differs from classical IC in terms of the nature of detection in that detection in IPC relates to properties of the eluent ion as opposed to the analyte. Specifically, the indirect detection arises from the reduction in the background UV absorbance of the mobile phase resulting from the displacement of a UV-active eluent ion with an eluted, UV transparent, analyte.

Since detection relies on an eluent characteristics, it is universal in the sense that any eluted analyte can produce a detector response. For truly transparent strong acid analytes, such a detection strategy allows for "standardless" calibration.[3,4] That is, the detector response per equivalent of the analyte is independent of analyte identity and thus a calibration obtained for one analyte in a particular mobile phase is applicable to other such analytes in the same mobile phase. If the analyte charge is known, its molar concentration can be determined with such a strategy even if its identity is unknown.

In most common applications of IPC, a weak acid is used as the eluting entity. Thus mobile phase pH will impact both the elution and detection characteristics of strong acid analytes because the speciation of the mobile phase is pH dependent. Since the nature of the strong acid analyte is not effected by changing mobile phase pH, the impact of the changing mobile phase pH can readily be estimated and mathematically modeled.[5-7]

For weak acid analytes, however, the effect of mobile phase pH on elution and detection characteristics is complicated by the fact that the speciation of the analytes also change as a function of mobile phase pH. Thus while the effect of mobile phase pH on the elution[8-10] and detection[10] of weak acid analytes has been studied, correlation of the observed trends with speciation properties of the analyte ions is complicated by simultaneous eluent ion and analyte ion changes.

In this study, the effect of mobile phase pH on the speciation of the eluent ion is minimized via use of an eluent (salicylic acid) whose speciation is not strongly affected by pH over the desired evaluation range. Thus the effect of analyte speciation on its elution and detection could be examined independently.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an Applied Biosystems (Ramsey, NJ) Model 400 pump, an electronically actuated Rheodyne (Cotati, CA) injector, an Alcott (Norcross, GA) Model 728 autosampler, an ABI Spectroflow 757 UV detector, a strip chart recorder, and a Hewlett Packard (Palo Alto, CA) HP3357 LAS computer data system. Ultraviolet spectra were obtained via a Hewlett Packard HP8452A photodiode array spectrophotometer. The separation were performed on a Waters (Bedford, MA) IC-PAK® anion column (50 x 4.6 mm).

Procedure

Mobile phases were prepared to contain 1 mM salicylic acid, adjusted to pH values of 3.50, 4.00, 4.50, 5.02, 5.44, 5.98, and 6.50 with 0.1 N sodium hydroxide and filtered through 0.45 μm media. Analytes investigated included chloride, nitrate, propionate, malonate, phosphate, acetate, and formate. Analyte standards containing approximately 0.2 meq/L of one or more of the analytes were prepared by diluting concentrated stock solutions in each mobile phase examined. In this way, sample and mobile phase pH were matched prior to sample injection.

The UV absorbance spectrum of each mobile phase was obtained over a range of 200 to 300 nm using a 1-cm quartz cuvette and water as the reference. Based on this analysis, a detection wavelength of 275 nm was chosen. The chromatograph was operated at a flow rate of 1.0 mL/min and with an injection size of 20 μL. The combination of analyte concentration and injection size was chosen to provide adequate analytical sensitivity without column overload and peak shape distortion. The mobile phases were changed in a random fashion, and after sufficient equilibration, triplicate injections of each standard mixture were made for each mobile phase. The resulting chromatograms were processed to obtain accurate analyte elution times and detector responses (peak area).

Calculations

The speciation of the analytes (fraction of the analyte that exists in its various charged forms) was determined at each mobile phase pH based on the

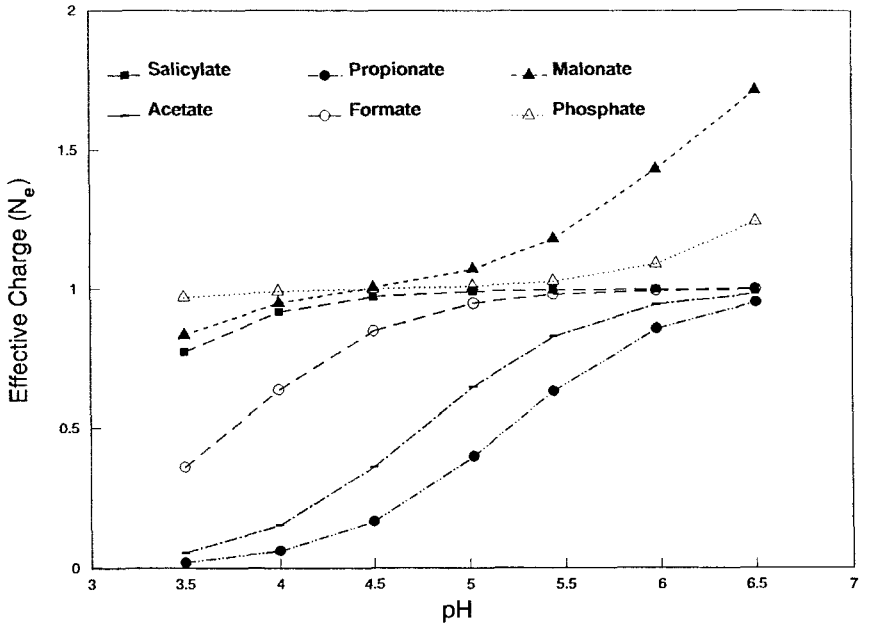

Figure 1. Effective Charge of the Pertinent Anions as a Function of Mobile Phase pH. While the N_e of the eluent ion (Salicylate) remains nearly constant over the pH range studied, N_e for the weak acid analytes changes significantly.

Table 1

Acid Dissociation Constants and Net Effect Charges for the Ions of Interest

Ion	pK_a*	Net Effective Charges (N_e) at a Given pH						
		3.50	4.00	4.50	5.02	5.44	5.98	6.50
Salicylic acid	2.96, 13.4	0.776	0.916	0.972	0.991	0.997	0.999	1.000
Acetate	4.75	0.053	0.151	0.360	0.651	0.830	0.944	0.983
Formate	3.75	0.360	0.640	0.849	0.949	0.980	0.994	0.998
Propionate	5.20	0.020	0.059	0.163	0.398	0.635	0.858	0.952
Malonate	2.80, 6.10	0.836	0.949	1.005	1.071	1.177	1.431	1.715
Phosphate	2.0, 4.0, 12.0	0.970	0.991	1.000	1.009	1.026	1.087	1.240

* pK_a values are from Reference 11.

mobile phase pH and the analyte's pK_a (Table I). Since the speciation of the mobile phase was nearly constant over the pH range observed, speciation calculations were performed without ionic strength corrections. The net effective charge (N_e) of the analytes was calculated based on the analyte's speciation:

$$N_e = \sum (z_i \times f_i)$$

where z_i is the charge of a particular form of the analyte and f_i is the fraction of the analyte that exists as that form at the pH of interest. The summation is taken over all possible forms of the analyte. Thus for example, N_e for phosphate was determined as

$$N_e = (1 \times f_{-1}) + (2 \times f_{-2}) + (3 \times f_{-3})$$

RESULTS AND DISCUSSION

The first issue addressed was to identify an appropriate eluent. For the purpose of this study, the eluent ion must fulfill three requirements: 1) it must have a strong UV absorption; 2) it must not change speciation appreciably over the pH range of interest (3.5 to 6.5) and 3) it must have sufficient eluting power (affinity for the stationary phase used) that workable separations can be obtained. While common eluents used in non-suppressed IC and IPC (such as phthalate, carbonate, hydroxide, borate/gluconate, hydroxybenzoic acid) fail to meet one or more of these requirements, salicylic acid was found to be an appropriate candidate. With pK_a values of 2.96 and 13.4, its speciation in the range pH range of 3.5 to 6.5 is dominated by the monovalent anion (see Table 1 and Figure 1 for its calculated N_e values). It contains a strong UV chromophore whose absorption spectrum is such that many detection wavelengths are possible.

Additionally, preliminary experiments indicated its affinity for the stationary phase used was such that viable separations could be achieved at analytically reasonable mobile phase concentrations.

The second issue addressed was the choice of detection wavelength. Two detector response issues are pertinent. The first issue addresses the ability of the UV detector to accommodate the indirect methodology. Since the background absorbance of the mobile phase may be considerable, the choice of the detection wavelength is not straightforward.

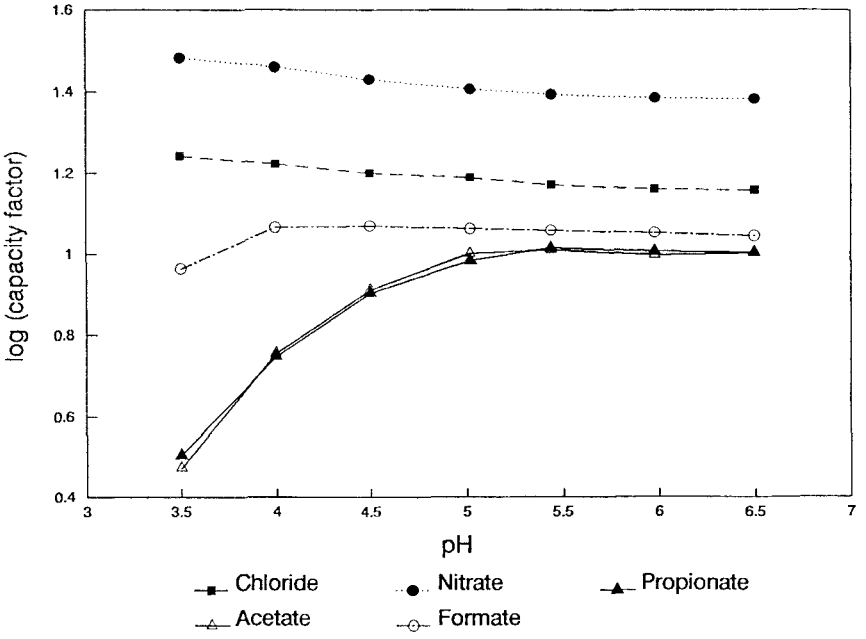

Figure 2. Effect of Mobile Phase pH on the Elution Characteristics [log (capacity factor)] of the Monovalent Analytes. While the retention of the strong acid analytes (chloride and nitrate) are only minimally affected by mobile phase pH, the retention of the weak acids changes significantly in response to their changing speciation as reflected in their N_e values.

Utilization of the absorption maximum does not necessarily produce the greatest detector sensitivity since the larger absolute magnitude of the analyte response is offset by an increased baseline noise. An additional practical issue to address is whether the absorption of the mobile phase at the wavelength of interest is so large as to saturate the detector.

The second detector wavelength issue addresses the need for the detector baseline signal to be constant over the pH range studied since changes in the mobile phase background absorbance could translate into a inherent mobile phase dependent sensitivity bias. In general, it is appropriate to work at the eluent ion's isobestic point. A detection wavelength of 275 nm meets the objectives of this research. This wavelength couples adequate sensitivity, detector compatibility and lack of pH-induced absorbance changes.

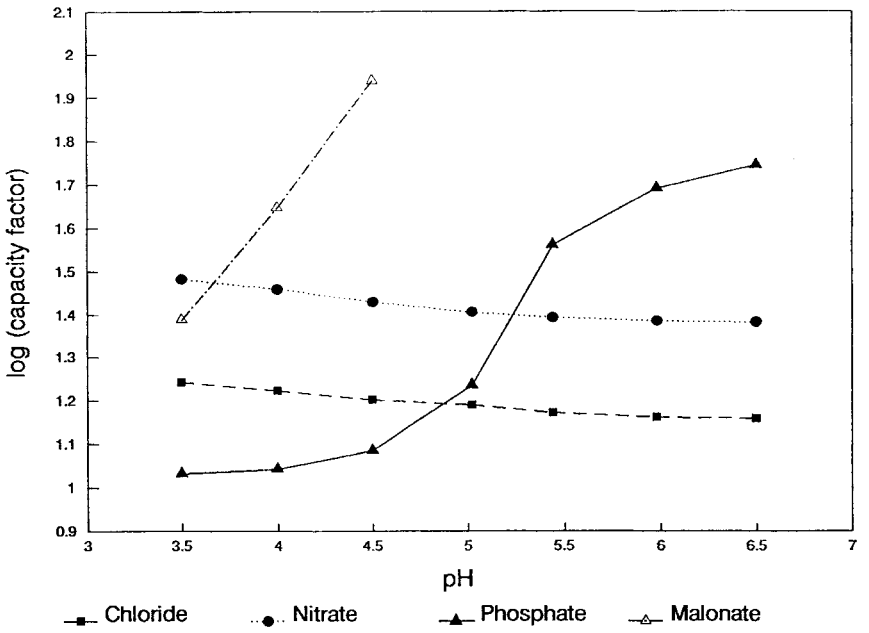

Figure 3. Effect of Mobile Phase pH on the Elution Characteristics [log (capacity factor)] of the Divalent Analytes. The capacity factor for phosphate changes significantly as the speciation of this ion changes from one dominated by the -1 ion to one in which the -2 ion is important.

The analytes examined included strong acids (chloride and nitrate) and monoprotic (acetate, formate, propionate) and multi-protic (malonate, phosphate) weak acids. The strong acids provide an assessment of elution and detection properties other than those related specifically to analyte speciation since their charge is constant. In the absence of pH effects on the eluent's background absorption or eluent strength or on column characteristics (e.g., ion exchange capacity), the detector response and capacity factor for the strong acids should be independent of pH. As shown in Figures 2 and 4, for the strong acids the relative detector response factor [ratio of the peak area at a particular mobile phase pH versus the peak area at the highest pH (6.5)] increases somewhat with increased mobile phase pH while the capacity factor decreases somewhat with increased mobile phase pH. At the lower pH values where these effects are most prominent, they are ascribed to small increases in the eluent ion's N_e. For example, between pH 3.5 and 4.5, the N_e of salicylic acid increases from 0.78 to 0.97.

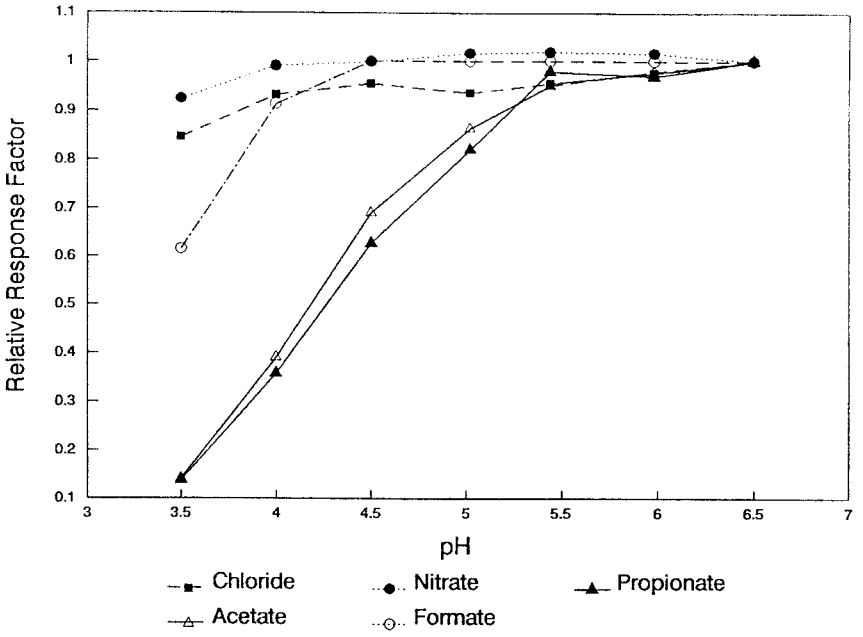

Figure 4. Effect of Mobile Phase pH on the Detector Response of the Monovalent Analytes. The relative response factor is the ratio of the peak area response obtained at a specific pH versus that response obtained at a pH of 6.50. The detector response of the weak acid analytes changes significantly as the N_e of these analytes increase with increasing mobile phase pH.

This change produces a stronger mobile phase resulting in somewhat lower capacity factors and relative responses. However, above a pH of 4.5, the consistency in the N_e for salicylic acid is reflected in stable capacity factors and relative responses. Thus at pH values above 4.5, changes in mobile phase pH produce no significant perturbations in the chromatographic system's detector responsiveness or column-related elution properties.

As observed in Table 1 and Figure 1, the weak acid analytes exhibit a wide range of N_e over the mobile phase pH range examined. Over this pH range, the speciation of both acetate and propionate change from one dominated by their uncharged form at low pH to one consisting almost solely of the -1 form at the high pH endpoint. Since the pK_a for formate is somewhat lower than those of acetate and propionate, its change in speciation is somewhat smaller over the pH range studied. However, its speciation changes significantly between a pH of 3.5 and 5.0.

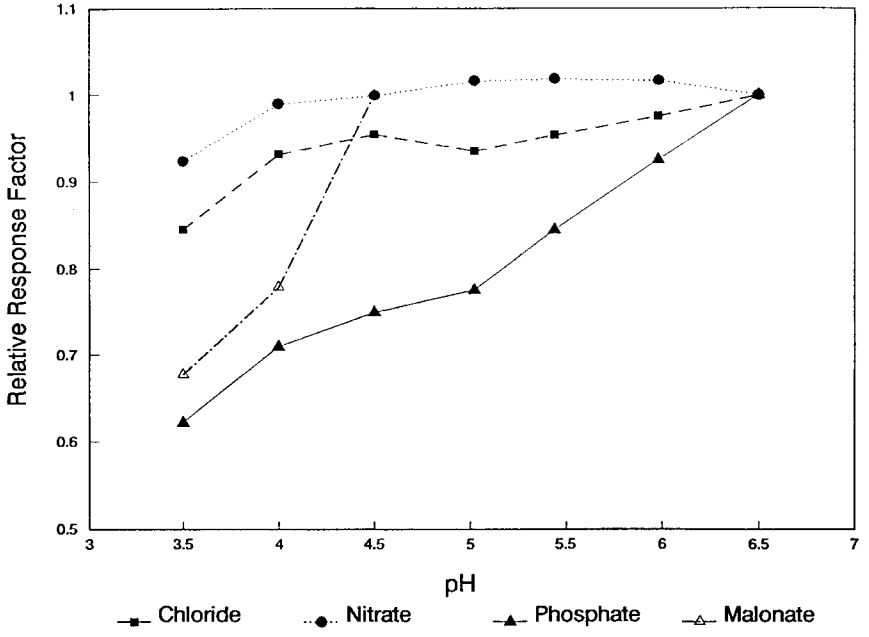

Figure 5. Effect of Mobile Phase pH on the Detector Response of the Divalent Analytes. The relative response factor is the ratio of the peak area response obtained at a specific pH versus that response obtained at the highest pH for which a response was obtained. The detector response of the weak acid analytes changes significantly as the N_e of these analytes increase with increasing mobile phase pH.

Thus one anticipates that the detection and elution properties of these analytes would change significantly over the pH range studied. Such behavior is shown in Figures 2 and 4. As N_e increases for all three analytes, the capacity factor increases (that is, the ions exhibit a stronger affinity for the stationary phase). Concurrently, as N_e increases, the relative response ratio increases as well. As N_e increases, the analytes displace a proportionally larger amount of the eluent ion (as dictated by electroneutrality) and the detector response increases. The similar change in N_e as a function of mobile phase pH for acetate and propionate (Figure 1) results in very similar capacity factor and detector response profile for these analytes (Figures 2 and 4).

The behavior of the two multi-protic analytes (phosphate and malonate) in response to changing mobile phase pH is shown in Figures 3 and 5. Malonate is such a strongly retained analyte that at pH values above 4.5 it cannot be eluted within a reasonable analysis time (less than 30 minutes) and as a

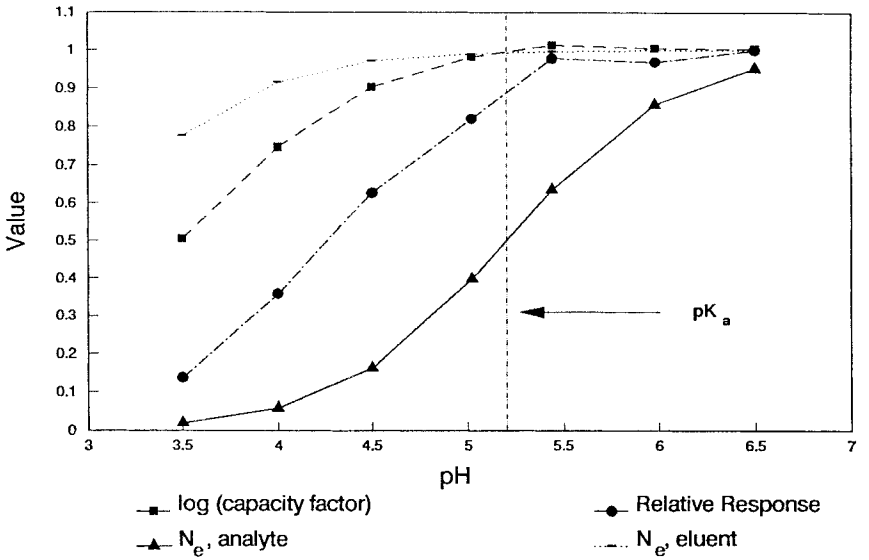

Figure 6. Effect of Changing Mobile Phase pH on the Detection and Elution Properties of the Propionate Ion. Extrapolation of the retention and elution trends to the pH at which their slope becomes zero provides an estimate of the ion's acid dissociation constant.

sufficiently narrow peak to allow for accurate quantitation. However, the change in elution and detection behavior of phosphate clearly mimics its change in speciation (from monovalent to divalent) and N_e over the pH range examined.

The relationships between relative response and capacity factor and N_e provides the ability to estimate an analyte's pK_a. Such an estimation is illustrated in Figure 6 for propionate. The pK_a is clearly defined in the N_e versus pH plot as that point where N_e equals 0.5. Additionally, both the relative response and capacity factor versus N_e plots will achieve a slope of 0 at a pH near the pK_a of the analyte of interest. As shown in Figure 6, both plots achieve the 0 slope value at a pH of between 5.00 and 5.44. Thus, within the resolution afforded by the mobile phases used in this study, the pK_a of propionate is accurately estimated. Similar success at estimating the pK_a values for the other analytes were obtained via examination of the pertinent plots for these analytes.

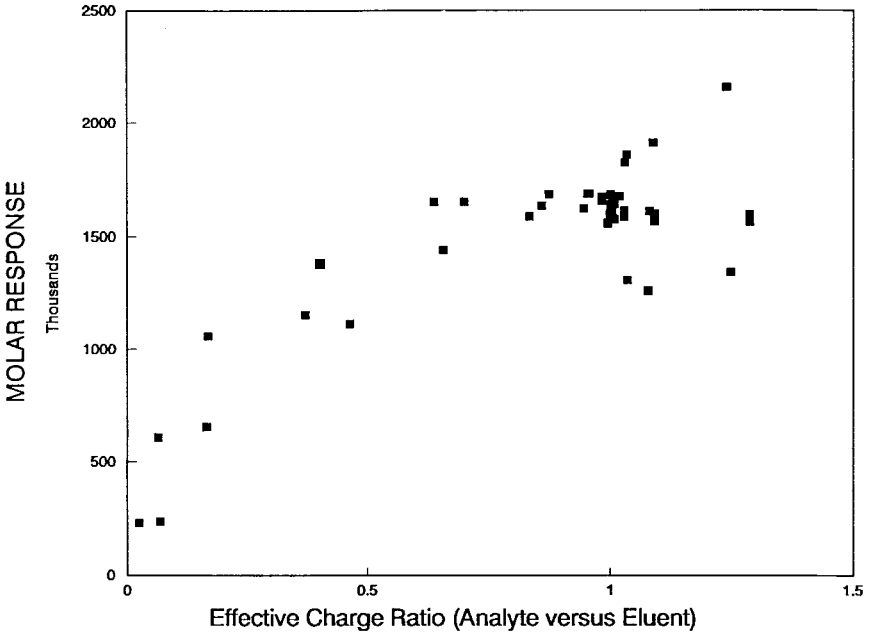

Figure 7. A Plot of Molar Response for all Analytes in all the Mobile Phases Examined versus the Effective Charge Ratio (analyte versus eluent). While the magnitude of the molar response increases with increasing effective charge ratio, the relationship is not strictly linear as one would expect from electroneutrality.

The observed effect of N_e on analyte elution and detection can be reconciled with a fundamental understanding of the nature of these processes. In the case of detection, the magnitude of the decrease in the background absorption signal (the molar detector response, D_r) must be equal to the product of the number of moles of the eluent ion displaced by the analyte (D_a) and the eluent ion's molar absorptivity (ε). The number of moles of eluent ion displaced by an analyte (D_a) is the analyte's molar concentration (C_e) times the net effective charge ratio of the analyte and eluent at a particular mobile phase pH:

$$D_a = (N_{e,a}/N_{e,e}) \times C_e$$

Thus the magnitude of the detector response can be expressed as:

$$D_r = (N_{e,a}/N_{e,e}) \times C_e \times \varepsilon$$

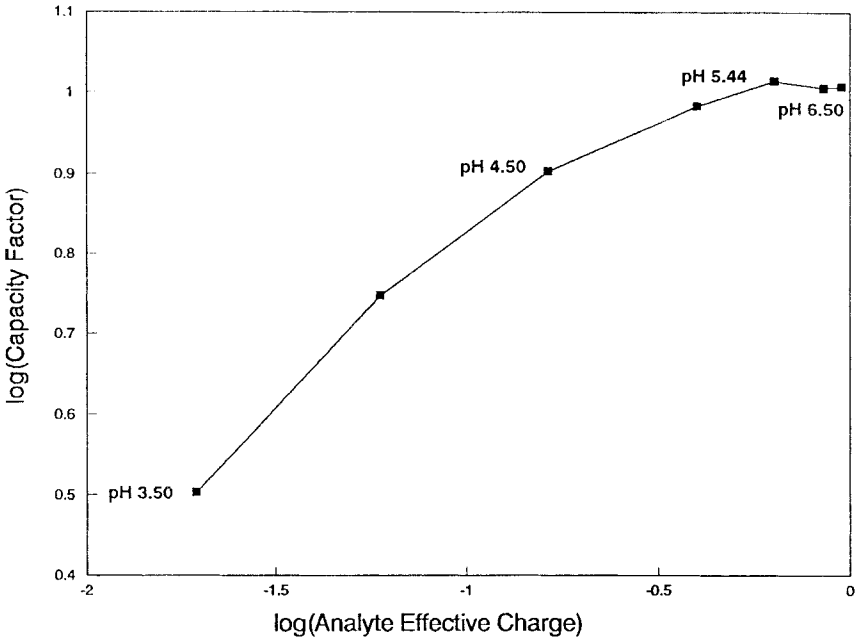

Figure 8. Log-Log Plot of Capacity Factor Versus Effective Charge for Propionate. As expected from ion exchange equilibria, the log-log relationship is roughly linear in the region of changing analyte effective charge. However, as the mobile phase pH increases well past the analyte's pK_a, the effective charge no longer increases and retention remains constant in the absence of eluent ion changes.

Since C_e was constant for each analyte over the entire pH range studied and if ε is independent of pH, then the plot of D_r versus should be linear with a zero intercept. The D_r versus effective charge ratio plot for all the data generated in this study is shown in Figure 7. The large number of data points in the region around the charge ratio of 1 reflects the data for the strong acid analytes and is indicative of the experimental uncertainty in terms of measuring the magnitude of the molar response. For the most part, the relationship between molar response and effective charge ratio is direct, with increasing charge ratio being reflected in a larger molar response. The relationship has a zero intercept, consistent with the no analyte, no response expectation. However, the pictured relationship is not linear as was anticipated by the above discussion. This deviation from the expected behavior may reflect a somewhat variable ε as a function of mobile phase pH or may suggest the presence of secondary column/analyte interactions during analyte elution.

Considering the retention elution behavior observed, the elution of a uniformly charged analyte (charge y) by a uniformly charged eluent ion (charge x) can be mathematically described by the following relationship (**1**):

$$\log (\text{retention parameter}) = \text{constant} - [(y/x)(C_e)]$$

where C_e is the concentration of the eluent ion. In this study, Ce is roughly constant over the pH range studied and both y, and to a lesser extant x, vary as a function of pH. Thus one anticipates that the log(capacity factor) and log ($N_{e,a}$) for the analyte would be roughly linear over the pH region, in which significant changes in $N_{e,a}$ is occurring.

Figure 8 shows the appropriate plot for propionate, which is similar to those obtained for the other mono-protic weak acids. With a pK_a of 5.2, the N_e for propionate does not change materially above a pH of 6.0 and thus the plot achieves a zero slope. Below a pH of 6.0, N_e changes significantly as a function of mobile phase pH and produces a roughly proportional change in log (capacity factor).

An equivalent plot for a multi-protic analyte (phosphate) is shown in Figure 9. A transition in elution properties is observed over the pH region of 4.0 to 5.5, reflecting a change in phosphate speciation from one dominated by the monovalent anion to one in which the divalent anion plays an important role in analyte behavior.

The fact that the transition occurs when the proportion of the phosphate ion which exists in the divalent form is very low (at pH 5.44, the net effective charge is only 1.03) reflects the much higher stationary phase selectively for the divalent anion versus the monovalent anion.

CONCLUDING COMMENTS

The examination of the effect of mobile phase pH on the elution and detection of weak acid analytes in indirect photometric chromatography is facilitated by the use of an eluent (salicylic acid) which exists with essentially a single effective charge over the pH range examined. Under such conditions, both the detector response and elution characteristics of weak acid analytes can be expressed in terms of the net effective charge exhibited by the analyte as a function of mobile phase pH. However, deviations of observed behavior from that anticipated considering a fundamental analysis of ion exchange and

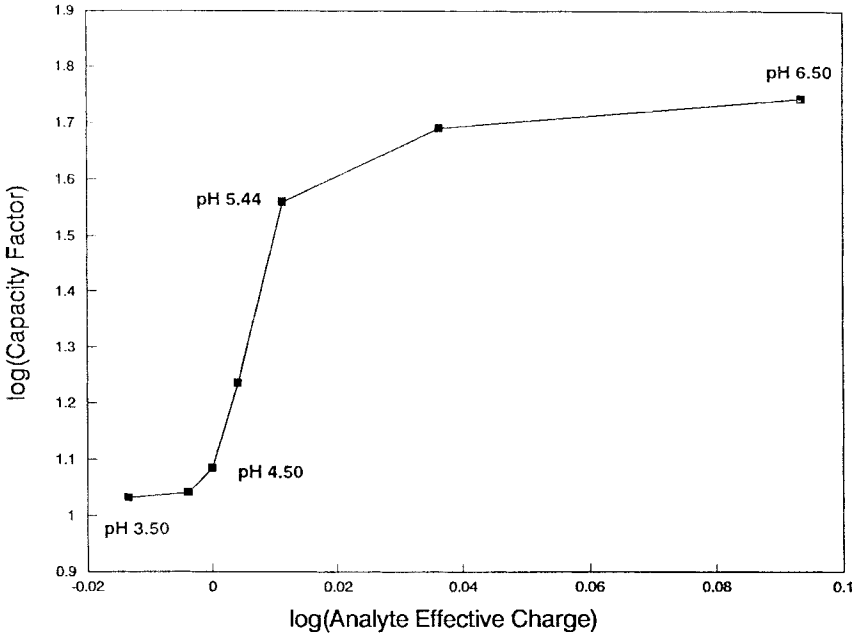

Figure 9. Log-Log Plot of Capacity Factor Versus Effective Charge for Phosphate. As expected from ion exchange equilibria, the log-log relationship is roughly linear in the region of changing analyte effective charge. However, the large break in slope observed represents the changing influence of the -1 and -2 charge forms of this analyte (and their greatly different stationary phase selectivity).

solution phase equilibria suggest that such an analysis does not account for all system changes that occur as a result of changing mobile phase pH. Despite these deviations, plots of retention and detection behavior of a weak acid analyte as a function of mobile phase pH can be used to estimate the acid's dissociation constant (pK_a).

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