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Role of additives in packed column supercritical fluid chromatography: suppression of solute ionization

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ABSTRACT

Tailing of polycarboxylic acids in packed column supercritical fluid chromatography (SFC) is primarily due to solute ionization. Very acidic additives improve the peak shape of acidic solutes by suppressing solute ionization. Coverage of active sites appears to be a secondary function of additives. The sorption of acidic additives was measured on five stationary phases used in packed column SFC. Surface coverages (at constant mobile phase composition) varied by more than 50-fold (0.4 to 21%), depending on the stationary phase identity. Within the group of additives used, coverage was independent of additive identity or concentration but was inversely proportional to modifier concentration. Chromatographic peak shapes of polyfunctional acidic solutes were also observed under the same conditions as used for the surface coverage measurements. Solute peak shapes depended on the acid strength of the additives but were unrelated to the amount of additive retained on the column.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a transitional technique between gas chromatography (GC) and liquid chromatography (LC) in that the mobile fluid is a gas that solvates. The most widely used fluid, carbon dioxide, is similar to liquid pentane or liquid hexane in solvent strength [1–3]. The polarity of carbon dioxide can be increased by adding small amounts of a polar organic modifier, but modifiers that are miscible with carbon dioxide are also only moderately polar (*i.e.*, $p' = 5.1$ for methanol *vs.* 0 for pentane and 10.2 for water [4,5]). Solvatochromic dye studies suggest [6] that very polar compounds, such as trifluoroacetic acid (TFA), when added to modifiers can significantly increase the polarity of modified mobile phases. Small concentrations (*i.e.*, 10^{-4} *M*) of such very polar compounds improve chromatographic peak shapes [7–11] and elute solutes that are normally retained. Such small concentrations cannot be directly added to non-polar supercritical fluids. Instead, they are added to a modifier of intermediate polarity, such as methanol. We differentiate them from modifiers by calling them additives. Additives provide a key to the separation of more polar solutes by SFC.

While it has been demonstrated that additives affect retention and peak shapes, the mode of operation of additives is unknown, although many workers assume that additives function by covering active sites. If additives function by covering active

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sites, then the peak shape should be related to the amount of an additive retained and the intensity with which it is held. Such measurements have not been made. The effect of additive polarity on additive retention and solute chromatographic peak shape have not been systematically studied. In this work we measured the retention of a series of progressively more polar additives on a series of progressively more polar stationary phases, and compared the amount of additive retained and additive polarity with solute chromatographic retention and peak shape. The number of active sites was also determined and compared with the amount of additive retained.

EXPERIMENTAL

Instrumentation

The chromatographic system included a Hewlett-Packard (HP) Model 1082 liquid chromatograph, modified to pump carbon dioxide, and an HP Model 1050 pump for modifier addition. Flow-rates and modifier concentrations (%, v/v) are based on volumetric flow-rates at the pumps. The carbon dioxide pump head temperature was 4.0°C. Detection was effected with an HP Model 1050 UV–VIS photodiode-array detector with a high-pressure flow cell. A low-volume electronic back-pressure regulator, built in-house, was used to control the system pressure. Additive concentration is expressed in terms of its concentration in the modifier, not in the complete mobile phase.

Breakthrough experiments used the three-pump system shown in Fig. 1. All three pumps ran continuously. A modified HP Model 1050 pump delivered a fixed flow of carbon dioxide. One of the remaining Model 1050 pumps delivered a fixed flow of pure modifier while the other Model 1050 pump delivered the same flow of modifier containing a small concentration of a very polar additive. A switching valve allowed the flow from either one of the modifier pumps to be mixed with the carbon dioxide at the head of the column. The other modifier flow was added downstream of the column and detector but upstream of the back-pressure regulator so that both modifier pumps were always pressurized to nearly the same pressure. Switching the valve interchanged the two liquid flows. The baselines in the figures demonstrating breakthrough measurements appear noisy. This noise has many sources. Most of the additives have low molar absorptivities, producing little change in signal for a few percent change in modifier concentration. The detection wavelength was 210 nm, where both methanol and the additives adsorb. Most of the mixing apparatus used in chromatography was removed, allowing larger than normal short-term composition and pressure variations.

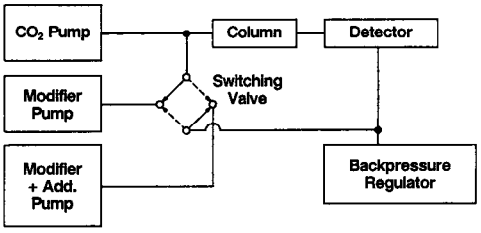

Fig. 1. Schematic diagram of instrumentation used for breakthrough experiments.

Columns

The columns were 100 mm × 2 or 2.1 mm I.D. standard packed columns for liquid chromatography (LC), which were washed overnight with pure methanol at 80°C before use. The 2 mm I.D. columns were purchased from Keystone Scientific (Bellefonte, PA, USA). The Phenyl and Cyanopropyl (CN) phases were on 5-μm Hypersil. The sulfonic acid (SA) packing was 5-μm Nucleosil. The Diol packing was 7-μm Nucleosil. The MOS (C_8) columns were 100 mm × 2.1 mm I.D. with 5-μm Hypersil, packed by Hewlett-Packard.

Column pretreatment

Two different column preparations were used, a methanol wash and a base wash. The methanol wash involved flushing the column with a low concentration (usually 4 or 6%) of methanol (without an additive) in carbon dioxide, for some fixed time (usually 20 min). After such washes, the additive was re-introduced into the mobile phase while keeping the modifier concentration constant. The time between the re-introduction of additive at the column inlet and the appearance of the additive in the column effluent constitutes a breakthrough time proportional to the amount of additive retained on the column. Methanol washes probably remove only weakly retained additives.

The base wash consisted of 10 ml each of (1) 1% N-*tert.*-butylammonium hydroxide in methanol, (2) methanol, (3) 1% acetic acid in methanol and (4) methanol; all four wash fluids contained 10% carbon dioxide. Base washes were followed by a methanol wash before breakthrough measurements. Base washes probably produce a "bare" column with no strongly adsorbed acidic additives.

Chemicals

Carbon dioxide was of supercritical grade in aluminium cylinders, purchased from Scott Specialty Gases (Plumsteadville, PA, USA). Methanol was of high-purity grade from Burdick & Jackson Labs. (Muskegon, MI, USA). Additives were purchased from Eastman Kodak (Rochester, NY, USA). Other chemicals were purchased from Aldrich (Milwaukee, WI, USA).

RESULTS AND DISCUSSION

Determination of active sites

There are *ca.* 8 μmol/m^2 of silanol groups on hydrolyzed bare silica particles [12–14]. The theoretical pH of silanol in water is 7.1 [15,16], but experimentally measured values can be much higher or lower [17]. Up to 5% of the total silanols are chemically distinct with a lower pH [18]. Other investigators have suggested that the fraction of more "reactive" silanols is less than 0.1–0.3%, which correlates with the concentration of metal ions in the silica [19–21]. Bonded stationary phases typically cover 40–60% of silica surfaces [22–24], so the total available silanol concentration would be reduced to 3.2–4.8 μmol/m^2, and reactive silanols would be reduced to 0.04–0.18 μmol/m^2. The total surface areas of several of the columns were measured using a modification [25] of tracer pulse chromatography [26]. The 300 Å Diol column had a measured surface area of 15 m^2 and the 4000 Å Diol column 3.0 m^2.

Breakthrough experiments

Column transit times were *ca.* 0.15 min, but as long as 45 min passed with no measurable additive in the column effluent after additives were introduced into the mobile phase at the column inlet. After such delays, the baseline undergoes a step change to a higher absorbance, as seen in Fig. 2. These breakthrough times, the flow-rate and the concentration of additive indicate the moles of additive retained on the column. Such breakthrough times, from each type of wash, could be reproduced to within *ca.* ±5% within one day and *ca.* ±20% over several months.

The amounts of additive retained on various columns are presented in Table I. Loading factors (μmol/m^2) were obtained by dividing the number of micromoles retained by the measured surface areas of representative columns. Loading factors were converted to surface coverage (%) by dividing by the total surface concentration of silanols on an uncoated packing (8 μmol/m^2) and multiplying by 100. A 100% coverage would be equivalent to a monolayer of additive on both bonded phase and exposed silica.

Loading factors and surface coverages are presented in Table II. Surface coverage was independent of the additive identity or acid strength, as similar coverages were measured using trifluoroacetic acid, dichloroacetic acid and citric acid. The retention of an additive is a strong function of stationary phase identity.

On the least polar columns (*i.e.*, MOS) the amount of additive retained was about the same after both the base wash and the methanol wash (the additive is easily removed), and the absolute amount was very small. The additive surface coverage of 0.4% was much lower than most estimates of the surface coverage of silica by all

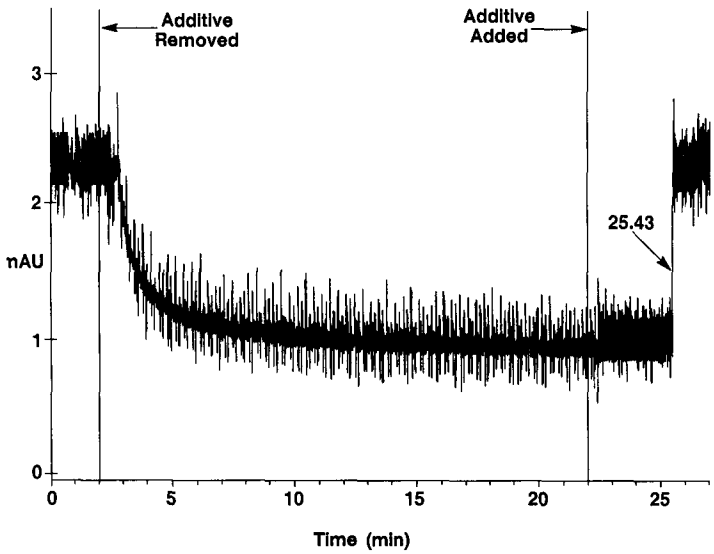

Fig. 2. Results from a typical breakthrough experiment. From 0 to 2 min: 6% (v/v) methanol containing 0.1% dichloroacetic acid in carbon dioxide; 2 to 22 min, 6% methanol in carbon dioxide (methanol wash); 22 to 27 min, 6% methanol containing 0.1% dichloroacetic acid, in carbon dioxide. Breakthrough time = 3.43 min. Column, 100 mm × 2 mm I.D., 7-μm Nucleosil, 4000 Å Diol, flow-rate 1 ml/min (at pumps; CO_2 at 4°C), 40°C, 207 bar.

TABLE I

MICROMOLES OF ADDITIVE SORBED ON COLUMNS FROM METHANOL–CARBON DIOXIDE MIXTURES AT 40°C AND 200 BAR

Columns, 100 × 2 mm I.D.

Stationary phase	Additive	After base wash	20-min methanol wash	Other methanol wash
MOS (C_8), 120Å, Hypersil	DCA		0.622[a],0.500[b]	
	TFA		0.75[a],0.57[b]	
CN, 120 Å, Hypersil	DCA	0.493[a]	0.590[a] 0.60[b]	
SA, 300 Å, Nucleosil	DCA	3.88[b]	0.207[b] 0.200[a]	39 min, 2.07[b]
	TFA		1.97[b] 3.19[a]	
Diol, 300 Å, 15 m^2, Nucleosil	DCA	11.60[b]	4.23[b]	70 min, 7.69
		13.69[b]	5.91[b]	
Diol, 4000 Å, 3.0 m^2, Nucleosil	Citric acid	3.05[b]	1.60[b]	50 min, 2.07, 120 min, 2.16[b]
	DCA	5.10[b]	2.52[b]	
	TFA	4.88[b]	1.63[b]	36 min, 1.99[b]; overnight, 4.44[b]

[a] 4% methanol containing 0.1% additive.
[b] 6% methanol containing 0.1% additive.

silanols, but was higher than some estimates of surface coverages of "reactive" silanols (0.1–0.3%) [19–21] on uncoated silica. The surface coverage on a cyanopropyl (CN) stationary phase was the same as on the MOS column. On the SA column, surface coverages were 4–8 times higher. The 300 Å Diol column had up to a 30 times higher surface coverage than the MOS and CN columns. The right-hand column in Table I indicates that even very long (2–24 h) methanol washes did not wash off as much additive as a base wash (some of the additive is difficult to remove). The 4000 Å Diol column has a surface coverage about twice that on the 300 Å Diol column. Both Diol columns showed a substantial difference between methanol and base washes (possibly owing to two forms of retention).

From the least to the most retentive column, the surface coverage varied more than 50-fold, from 0.4% to 21%, from a $4 \cdot 10^{-4}$ M additive solution. As the MOS and CN columns did not retain much additive, the population of active sites must be

TABLE II

DATA FROM TABLE I FOR DCA ADDITIVE CONVERTED TO LOADING FACTORS AND SURFACE COVERAGE

Column	After base wash		After methanol wash	
	Loading factor (μmol/m^2)	Surface coverage (%)	Loading factor (μmol/m^2)	Surface coverage (%)
MOS			0.041	0.5[a]
			0.033	0.4[b]
CN	0.033	0.4[b]	0.033	0.4,0.6[a]
			0.048	
			0.040	0.5[b]
SA	0.26	3.4[b]	0.13	1.6[b]
Diol (300 Å)	0.84	12[b]	0.76	4.3[b]
Diol (4000 Å)	1.70	21[b]	0.76	10[b]

[a] 4% methanol.
[b] 6% methanol.

small, or the additives must not interact with active sites. The large differences in surface coverage between MOS and CN on the one hand, and Diol and SA columns on the other, indicate that additives are retained on the Diol and SA columns by some mechanism in addition to coverage of active sites.

The surface coverage by additive appeared to be independent of the additive concentration in the mobile phase, as shown in Fig. 3. If the concentration on the stationary phase is fixed while the mobile phase concentration changes, then the additive distribution constant (K_D) changes and the additive does not partition like a solute. An adsorption isotherm relating surface coverage to additive concentration could not be generated either. In contrast, additive surface coverage was inversely proportional to modifier concentration. Stepwise increases in the concentration of the polar modifier (additive concentration also increases) produce step changes in background absorbance, with an additional peak or maximum (absorbance due to additive) superimposed on the step change, as shown in Fig. 4a. The areas of the peaks are measures of the amount of additive washed off the column by the increased concentration of the modifier. In Fig. 4a, the mobile fluid initially contained 4% of methanol in carbon dioxide, which was increased to 8% of methanol at 4 min and to 12% at 12 min.

Stepwise decreases in the concentration of modifier confirmed the inverse relationship between the modifier concentration and surface coverage. The effect was clearly seen when modifier concentration was decreased from 4% to 2%, as in Fig. 4b. Between 17 and 20 min (in Fig. 4b) the background absorbance was characteristic of 4% of modifier containing the additive. At 20 min, the modifier concentration was decreased to 2%. After a short delay, the background absorbance decreased but to a value substantially below that expected for 2% of modifier containing the additive.

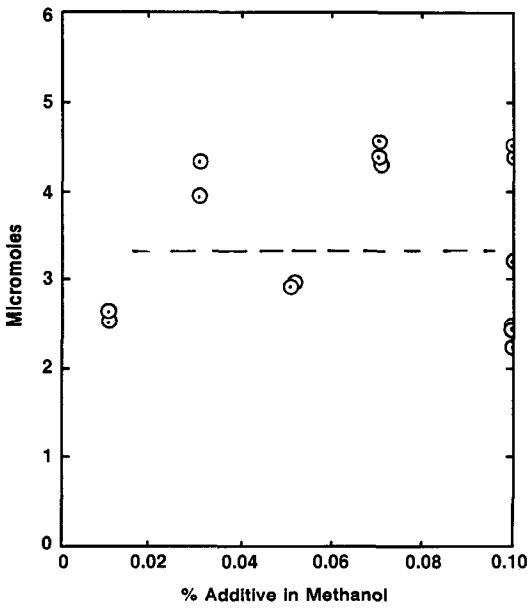

Fig. 3. Effect of additive concentration on the amount of additive retained. Dichloroacetic acid in 6% methanol after methanol washes; 40°C, 207 bar outlet pressure, a 100 mm × 2 mm I.D., 7-μm Nucleosil, 300 Å Diol column.

At about 39 min, the baseline increased to the expected value. The decrease in absorbance between 20 and 39 min indicates that most of the additive was being removed from the mobile phase and was being retained by the column. The depth and duration of the decrease in absorbance and the flow-rate provide a measure of the change in the amount of additive retained. The concentration of additive emerging from the column was calibrated against the absorbance of additive–modifier–carbon dioxide mixtures with the column removed. Table I gives the absolute amount retained at one modifier concentration. Table I and measurements on Fig. 4b produced the relationship between modifier concentration and amount of retained additive shown in Fig. 5.

Chromatographic experiments

Additive polarity. The additives and stationary phases used to measure surface coverage were also used to collect chromatograms of benzoic acid, 1,2-benzenedicarboxylic (phthalic) acid and 1,3,5-benzenetricarboxylic (trimellitic) acid. The solutes were separated on five stationary phases using five additives of increasing polarity. The columns were exposed to additives, then washed overnight with methanol at 80°C before equilibration with the mobile phases containing the additive. The methanol concentration was adjusted to obtain roughly the same retention times on all the columns. The concentration of additive was fixed at 0.2% in methanol. If the methanol concentration in the mobile phase is changed, the additive concentration also changes proportionally. Chromatograms obtained with MOS, Phenyl and CN columns are shown in Fig. 6. The Diol and SA columns are considered separately below.

The weakest acids (acetic and chloroacetic acid) were ineffective in improving

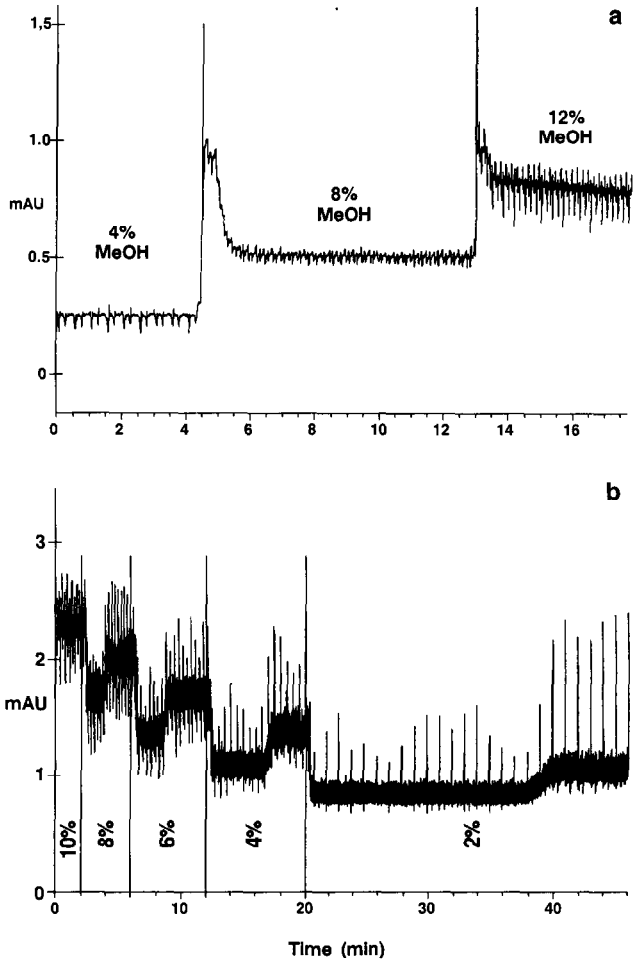

Fig. 4. Effect of step changes in modifier concentration. (a) Typical step up in concentration: 0.1% TFA additive in methanol (MeOH); total flow-rate = 1 ml/min, 40°C, 130 bar. A 100 mm × 2 mm I.D., 5-μm Nucleosil SA column. (b) Typical step down in concentration: 1.00 g/l citric acid in methanol; flow-rate = 1 ml/min, 40°C, 207 bar; a 100 mm × 2 mm I.D., 7-μm Nucleosil, 4000 Å Diol column.

peak shapes when used as additives. Dichloroacetic acid improved peak shapes on the CN column but not on the MOS or Phenyl columns. According to Tables I and II, the MOS and CN columns retain the same, small amount of additive. The poor peak shapes on the MOS column and the symmetrical peaks on the CN column, under identical conditions, suggest that there is a substantial difference in additive behavior on the two column types. The additive is easily washed off with a methanol wash so it is not strongly adsorbed.

Significantly higher modifier concentrations were required to obtain the same retention times on a sulfonic acid column as on the CN column, as can be seen by comparing Figs. 6 and 7. As on the CN column, only the more acidic additives

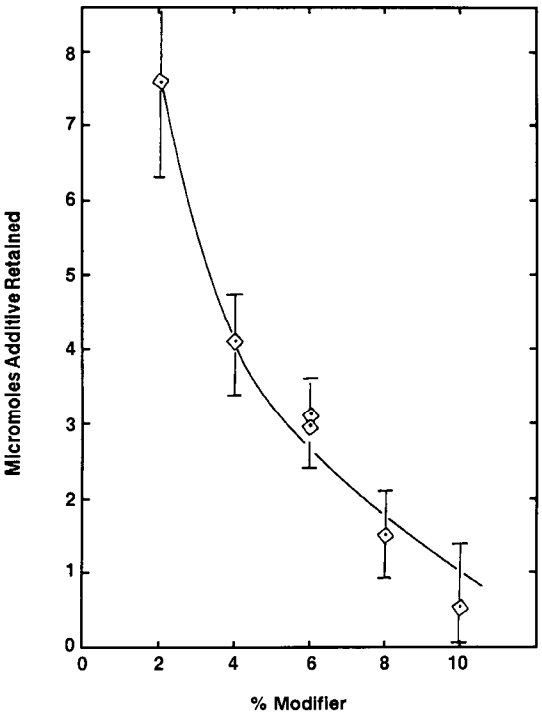

Fig. 5. Effect of modifier concentration on the amount of additive retained. Conditions as in Fig. 4b.

improved peak shapes. Sulfonic acids are strong acids, whereas silanols have a surface pH between 4 and 7 [20,21]. If the sulfonic acid functional groups are substantially more acidic, and are present in greater numbers than even the most "reactive" active sites, there should be only one dominant (solute) retention mechanism and polar solutes should not tail. However, the peak shapes were poor on the SA column, when no additive or a low polarity additive was used. This suggests a tailing mechanism other than interaction with active sites.

The chromatographic behavior of Diol phases is different from that on the other column types. On the 300 Å Diol column, phthalic acid and trimellitic acids were strongly retained, with poor peak shapes (not shown in Fig. 8) when acetic acid was used as the additive. The retention decreased dramatically, peak shapes improved and the elution order even reversed, with stronger acid additives, as shown in Fig. 8. Such dramatic changes in retention and selectivity are usually not associated with a simple coverage of active sites, and imply a major change in the chemical characteristics of one or both chromatographic phases.

Additive concentration. The retention of benzoic, hydroxybenzoic and benzenedicarboxylic acids was measured as a function of additive (citric acid) concentration, as shown in Fig. 9. Some citric acid was probably present on the column even when the mobile phase contained no added citric acid, as the column was prepared by washing overnight with pure methanol. The breakthrough experiments showed that additives produced high surface coverages on Diol columns but, once established, the

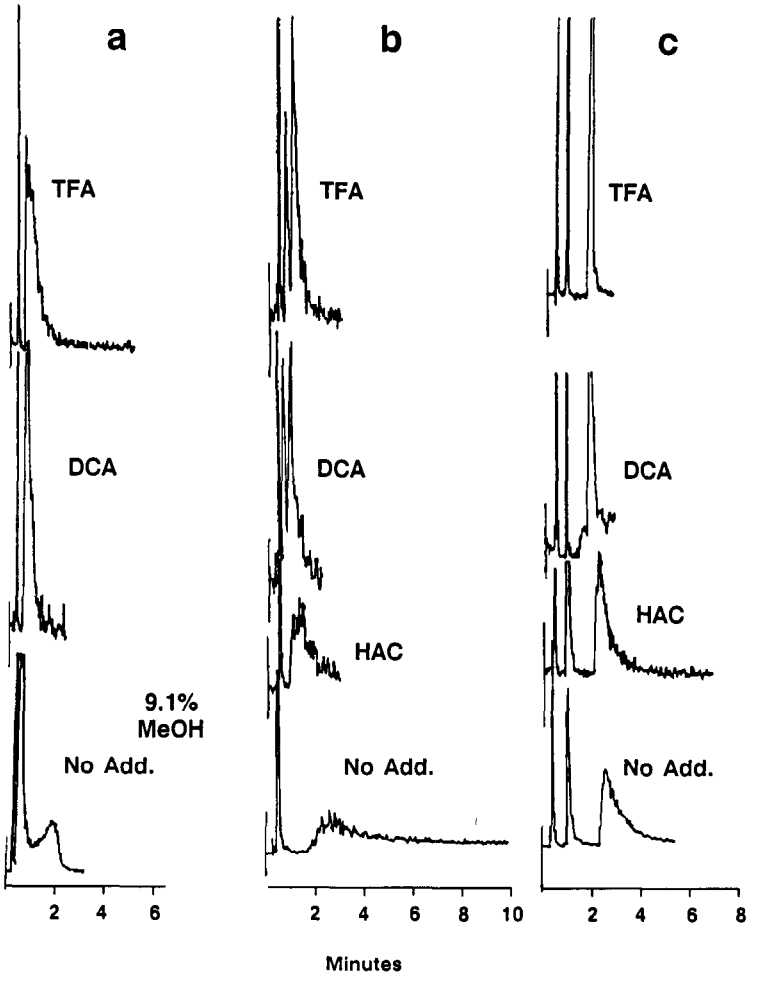

Fig. 6. (a) 1.2% methanol on MOS column; (b) 0.8% methanol on Phenyl column; (c) 1.6% methanol on CN column. Solute elution order: benzoic acid, phthalic acid and trimellitic acid. Additives: HAc = acetic acid; CA = chloroacetic acid; DCA = dichloroacetic acid; TCA = trichloroacetic acid; TFA = trifluoroacetic acid. Methanol (MeOH) contains 0.2% additive; in carbon dioxide. A 100 mm × 2 mm I.D. column; flow-rate = 3 ml/min, 40°C, 130 bar outlet pressure.

amount of additive present on the stationary phase is independent of its concentration in the mobile phase. The retention of the three hydroxybenzoic acids changed dramatically with the first small additions (from 0 to <0.1–0.2%) of citric acid to the mobile phase, whereas the retention of 1,3- and 1,4-benzenedicarboxylic acid changed only modestly. The retention and peak shapes of both 1,2-substituted solutes (salicylic and phthalic acids) changed most dramatically with the first addition of citric acid. More important, the retention of these two solutes continued to change as the additive concentration was increased. Solute separation factors ($\alpha = k'_2/k'_1$) *vs.* additive concentration, as shown in Fig. 10, indicate that the additive has an even larger

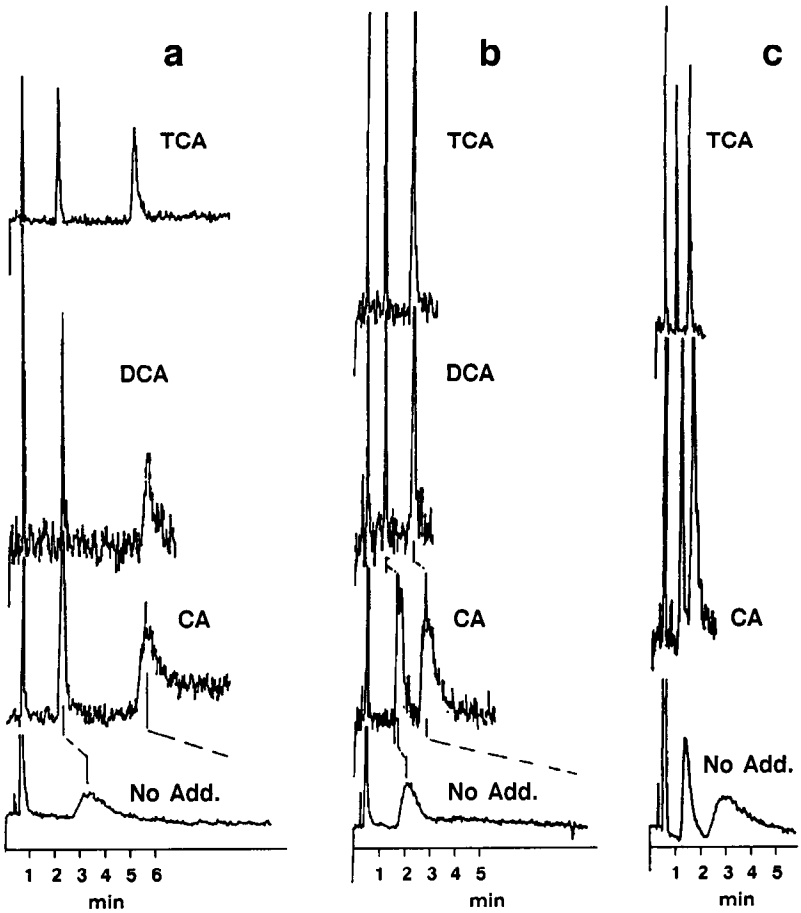

Fig. 7. Chromatograms as in Fig. 6, except obtained on an SA column. (a) 3.0% methanol plus additive; (b) 4.8% methanol plus additive; (c) 6.3% methanol plus additive.

impact on selectivity than is evident from Fig. 9. The smallest changes in selectivity occurred between similar compounds (*i.e.*, pairs of dicarboxylic acids). Some peaks were symmetrical even when there was no additive in the mobile phase (see Fig. 11). For those solutes, the first addition of the additive dramatically shifted the retention but did not change peak shapes. Phthalic acid did not elute without additive, and was severely tailed, using 0.05% citric acid in methanol ($2 \cdot 10^{-4}$ *M*) in the mobile phase. The peak shape improved as the additive concentration was increased.

Correlation with acid strength

A possible explanation for the poor peak shapes of carboxylic acids using modified fluids, and the improvements attributable to additives, involves partial ionization of such solutes. Several reports [8,10], have discussed ion-pair separations in supercritical fluids, so the concepts of ionization and ion-pair formation in supercritical fluids is not new. In LC, ion-pairing agents improve the peak shapes of some

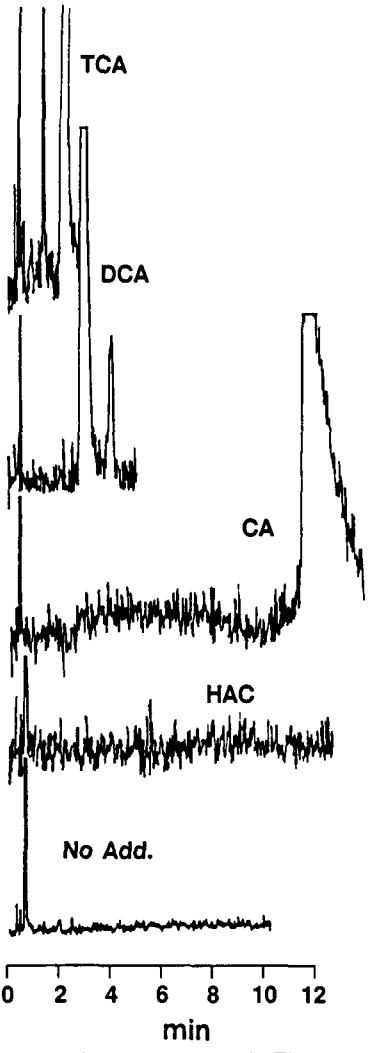

Fig. 8. Chromatograms as in Fig. 6, except obtained on a Diol column. 4.8% methanol plus additive.

aromatic carboxylic acids [27]. In this work, however, stronger acids were used to improve the peak shapes of less acidic solutes through ionization suppression. Such suppression also has precedents in LC [28,29].

In the absence of measured values in methanol–carbon dioxide mixtures, dissociation constants of acids in water [30,31] can be used to indicate at least a relative tendency for acids to ionize. Some dissociation constants in water and methanol are presented in Table III.

As shown in Figs. 6–8, the peak shapes of phthalic and trimellitic acid were always poor on all the stationary phases when no additive was present in the mobile phase. Only additives more acidic (lower pK_a values) than the solute improved the peak shapes. Acetic acid was ineffective, chloroacetic acid was partially successful and

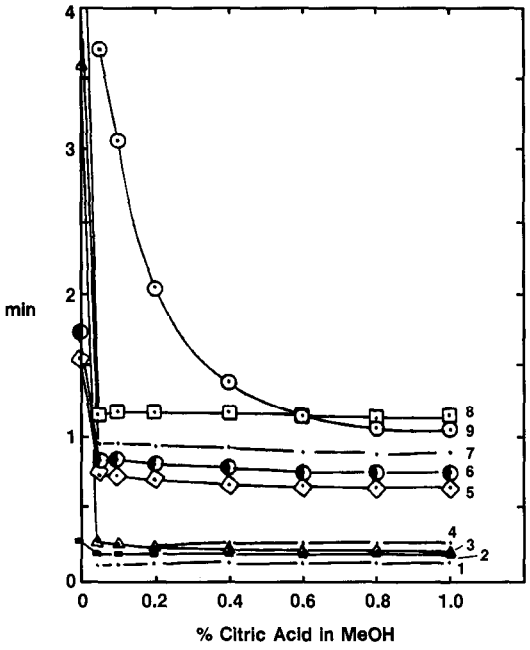

Fig. 9. Retention time *vs.* additive concentration. Lines 1 and 4 = trace contaminants; 2 = benzoic acid; 3 = 2-hydroxybenzoic acid; 5 = 1,4-benzenedicarboxylic acid; 6 = 1,3-benzenedicarboxylic acid; 7 = 3-hydroxybenzoic acid; 8 = 4-hydroxybenzoic acid; 9 = 1,2-benzenedicarboxylic acid. Mobile phase: 5.7% (v/v) methanol in carbon dioxide at 40°C; flow-rate = 3 ml/min, outlet pressure = 200 bar. Column: 100 mm × 2 mm I.D., 7-μm Nucleosil 300 Å Diol.

dichloroacetic acid dramatically improved the peak shapes on the SA column. According to Table III, acetic acid is much weaker ($pK_a = 4.75$), chloroacetic acid is of similar acid strength (2.85), while dichloroacetic acid is a stronger (1.48) acid than phthalic acid ($pK_{a,1} = 2.89$).

In Figs. 9–11, the retention of *ortho*-(1,2-)substituted solutes changed dramatically with citric acid concentration. The retention of the other solutes ceased to shift after the first small additions of citric acid. The first dissociation constant of the citric acid additive ($pK_{a,1} = 3.14$) is about the same as those of the *ortho*-substituted solutes [2-hydroxybenzoic acid ($pK_a = 2.97$) and 1,2-benzenedicarboxylic acid ($pK_a = 2.89$)], but more acidic than the other solutes ($pK_a = 3.51$–4.48). This suggests that additives suppress the ionization of less acidic solutes, but are only partially effective in suppressing the ionization of acids of similar strength.

CONCLUSIONS

Solute ionization is a major cause of peak tailing of carboxylic acid solutes in SFC. Such ionization can be suppressed by the common ion effect using additives that are more easily ionized than the solutes (*i.e.*, that are stronger acids). Tailing could not be suppressed simply by increasing the polarity of the mobile phase or by decreasing the polarity or activity of the stationary phase. Modifiers, such as methanol, were

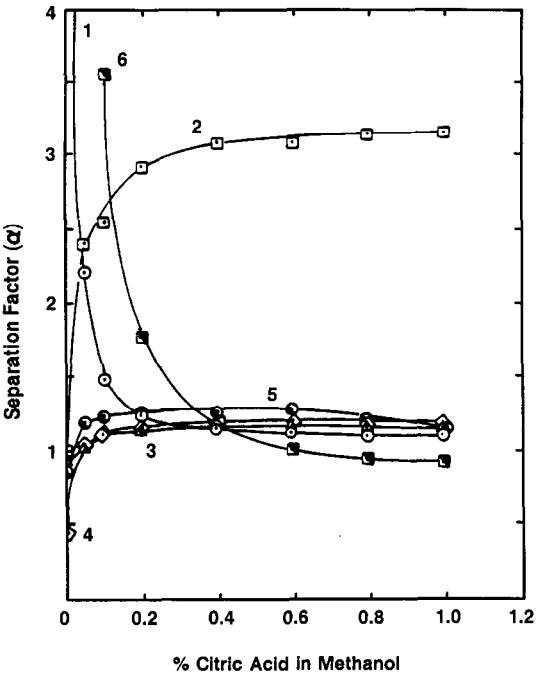

Fig. 10. Separation factors (α) *vs.* citric acid concentration for data in Fig. 9. Key: 1 = 3/2 in Fig. 9; 2 = 5/3 in Fig. 9; 3 = 6/5 in Fig. 9; 4 = 7/6 in Fig. 9; 5 = 8/7 in Fig. 9; 6 = 9/8 in Fig. 9.

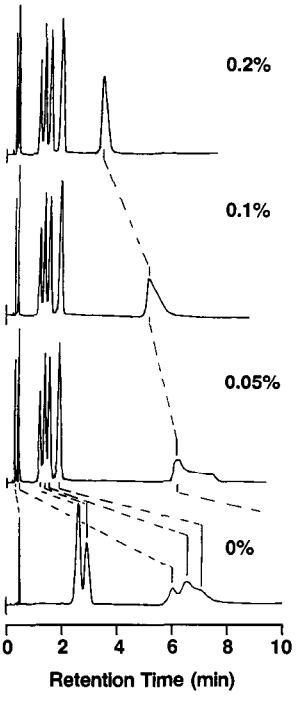

Fig. 11. Chromatograms obtained at a constant modifier concentration but with various additive concentrations. Conditions and elution order as in Fig. 9.

TABLE III

REPRESENTATIVE pK_a VALUES IN WATER AND METHANOL FOR VARIOUS ADDITIVES AND SOLUTES [30,31] INDICATING THE RELATIVE TENDENCY OF ACIDS TO IONIZE

	pK	pK_2	pK_3	pK_a (methanol)
Additive				
Acetic acid	4.75			9.52
Chloroacetic acid	2.85			
Dichloroacetic acid	1.48			
Trichloroacetic acid	0.70			
Trifluoroacetic acid	0.50			
Octanoic acid	4.89			
Citric acid	3.14	4.77	6.39	
Solute				
Benzoic acid	4.19			10.72 (ethanol)
2-Hydroxybenzoic acid	2.97	13.4		8.7
3-Hydroxybenzoic acid	4.06	9.92		
4-Hydroxybenzoic acid	4.48	9.32		
1,2-Benzenedicarboxylic acid	2.89	5.51		11.65 (pK_2)
1,3-Benzenedicarboxylic acid	3.54	4.60		
1,4-Benzenedicarboxylic acid	3.51	4.82		
2,3-Dihydroxybenzoic acid	2.94			
2,5-Dihydroxybenzoic acid	2.97			
3,4-Dihydroxybenzoic acid	4.48			
3,5-Dihydroxybenzoic acid	4.04			

not effective in suppressing tailing. Additives were only effective when they were stronger acids than the acidic solutes. However, even very acidic additives were ineffective in improving peak shapes on low-polarity columns such as C_8 (MOS). This implies that the column surface needs to be hydrophilic for additives to be effective. On polar columns, such as sulfonic acid or Diol, most of the additive retained appears to interact with the stationary phase, not the bare silica surface.

Polar columns exhibit as much as a 50-fold higher additive surface coverage than non-polar columns. However, both moderate- (CN) and high-polarity (SA or Diol) columns can produce symmetrical peaks for polar solutes. On the less polar columns, less than 1% of the surface is covered by "reactive" or "active" sites. These sites cannot be very polar as the additive can be washed off by a relatively mild methanol wash. Polar columns are unlikely to have 50-fold more active sites, so most of the additive retained must interact with the stationary phase, not active sites. Part of the retained additive is much more difficult to wash off, indicating a stronger interaction between the additive and the stationary phase than between the additive and "active" sites. In some instances, retention changes with additive concentration even through the surface coverage on the column apparently does not. Subtle differences in acid strength between additives and solutes appear to have a significant effect on solute peak shapes and retention.

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