

**RETENTION CHARACTERISTICS OF A pH TUNABLE WATER STATIONARY
PHASE IN SUPERCRITICAL FLUID CHROMATOGRAPHY**

by

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ABSTRACT

The retention characteristics of a novel pH tunable water stationary phase are presented. The method utilizes a change in mobile phase from N₂ to CO₂ to acidify the water phase *in-situ* and control the ionization and elution of organic acids. With N₂ present the phase pH > 5.4 and the acids are ionized and strongly retained. Conversely, with CO₂ present the pH < 3.8 and the acids are neutralized and can elute. This effect is reasonably independent of time. For example, at 80°C hexanoic acid readily elutes from a 10 m column after switching to CO₂ at any point over a 1 h period. Beyond this, however, some broadening and peak erosion is noted. Acids are also retained on 10 and 2 m columns similarly, since their elution primarily depends upon the change in stationary phase pH. Altering the CO₂ solubility in the water phase alone (i.e., through changing system temperature and pressure without using N₂) also produces similar changes in stationary phase acidity. However, this approach yields greater system noise and instability. The N₂/CO₂ switching mode is used to analyze organic acids in various samples and is found to provide high selectivity for them over other matrix components. Therefore, this approach can potentially simplify the analysis of such acids in complex samples.

Keywords: water; stationary phase; tunable; supercritical fluid chromatography; carbon dioxide

INTRODUCTION

Supercritical fluid chromatography (SFC) has long been established as a useful separation technique that is complementary to conventional high performance liquid chromatography (HPLC) and gas chromatography (GC) (1-8). The most common mobile phase in SFC is CO₂. This is mainly due to its mild critical parameters, inertness, environmental compatibility, available purity, low cost, and ability to interface with the desirable universal flame ionization detector (FID) (2-14). Similar to HPLC, the mobile phase in SFC is often altered during separations to influence analyte retention. For example, this can be done by adding a polar solvent like methanol to the non-polar CO₂ to improve analyte solubility, analogous to HPLC (2, 4, 6, 7, 10). Similarly, as in GC, the column temperature can also be varied to adjust analyte elution times (4). Further though, and more specific to SFC, the CO₂ mobile phase pressure/density can also be changed to alter analyte retention on the column (4).

In contrast to this, an interesting trend over the last several years has been the emergence and development of a few examples of chromatographic methods that focus on dynamically changing the stationary phase properties during separations to affect analyte retention. For example, temperature responsive membranes (15) and light or solvent sensitive smart polymers (16) have been incorporated into stationary phase materials and shown to help control analyte retention through external manipulation of these parameters. As well, in a very interesting electrochemical approach, a conductive stationary phase has been used to control analyte retention through altering an external potential applied to the column (17, 18). Since such approaches can offer great advantages to separations through providing additional selectivity and control of analyte elution (15-18), their continued exploration and development is potentially very beneficial.

Recently we introduced a novel capillary column SFC-FID system that employs water as the

stationary phase and CO₂ as the mobile phase (19, 20). In this method, a water stationary phase is established on the inner wall of an otherwise empty and uncoated stainless steel (SS) capillary, providing an environmentally ‘green’ chromatographic coating that can be easily replenished. The phase is stable and stationary over a wide range of conditions, and provides useful normal phase separations of polar analytes. Specifically, the latter are usually quite well retained based largely on their relative water solubility, while non-polar analytes often show no retention. Additionally, this water stationary phase has also been employed for GC separations using N₂ as a carrier gas (21). In working with this water stationary phase system, we have discovered that organic acids will not elute in the GC mode. However, they readily elute when operating in the SFC mode. These observations presumably stem from the fact that the latter CO₂/water interface is thought to be acidic (22), whereas the former N₂/water interface should be pH neutral. If so, the acids are likely ionized in the GC mode, where they remain strongly partitioned in the water stationary phase and heavily retained on the column.

This behavior suggests that a hybrid system using both N₂ and CO₂ mobile phases could be potentially used to control the water stationary phase pH *in-situ*, and hence, analyte retention on the column as well. Here we explore this possibility and describe a novel pH tunable water stationary phase for the separation of such polar analytes. Specifically, through toggling between a N₂ and CO₂ mobile phase, the method provides unique separations where the elution of organic acid analytes is directly controlled by changing the stationary phase properties *in-situ*. The general operating features of the system are explored and presented. As well, the mechanism for this behavior is confirmed and discussed. Finally, the system performance is demonstrated and assessed in the selective analyses of several different samples.

EXPERIMENTAL

Instrumentation

Figure 1 presents a schematic diagram of the pH tunable water stationary phase apparatus. ISCO model 260D syringe pumps (ISCO, Lincoln, NE, USA) were used to deliver CO₂ and H₂O to the system. SS tubing (0.010" i.d. x 1/16" o.d.) was used to connect the various system components as follows. First, a shut off valve (HiP Taper Seal Needle Valve; Western Gauge and Instruments Ltd, Calgary, AB, CAN) and a Swagelok plug/vent valve (Calgary Valve and Fittings, Calgary, AB, CAN) were joined in-series with the CO₂ pump outlet to respectively stop CO₂ flow and vent excess CO₂ pressure from the system before changing to a N₂ mobile phase. Nitrogen gas was delivered through a Swagelok plug valve (Calgary Valve and Fittings) that also acted to stop flow as needed when switching the system to CO₂. The N₂ and CO₂ lines were then joined in a SS Valco T-union (Chromatographic Specialties, Brockville, ON, CAN). Tubing from the outlet of this union was led into a Shimadzu GC convection oven (model GC-8A; Shimadzu, Japan) where it joined to the H₂O line in another SS T-union. Here, the addition of a small flow of H₂O was used to humidify the mobile phase and help prevent evaporation of the water coating on the column. A 1 m length of tubing leading from this union then helped preheat the mobile phase to the oven temperature before briefly exiting and connecting to a 0.5 µL Valco Cheminert internal loop injector (Chromatographic Specialties).

A 10 m SS capillary (0.010" i.d. x 1/16" o.d.) used for separations was connected to the injector and routed back into the oven. The column outlet was led through the oven wall into a second Shimadzu GC-8A oven where it was connected with a fused silica restrictor (50 µm i.d. x 360 µm o.d. x 40 cm long) using a zero dead volume SS Valco union (Chromatographic Specialties). This oven was used to control the restrictor temperature independent of the column

and was normally held at 150°C. The restrictor led into the FID jet of the GC and was positioned 1.5 cm below the flame for smooth system operation. This fused silica restrictor was replaced as needed due to erosion caused by the heated water. Unless stated otherwise in the text, the operating parameters normally employed for separations were 60 atm CO₂, 9.5 atm (140 psi) N₂, and 1 µL/min H₂O (as detailed below). The FID was typically supported by 240 mL/min air and 45 mL/min H₂, and the detector was held at 300°C.

Operating Procedures

The water stationary phase was coated on the capillary column as described previously (19). In a typical chromatographic run, the system was established using a N₂ mobile phase and the sample was injected. After separating components under this neutral stationary phase condition for a designated amount of time, the N₂ flow was stopped while the CO₂ flow was simultaneously initiated. The remainder of the separation was then carried out under this acidified stationary phase condition. Upon completion, the excess CO₂ pressure was relieved from the system before switching back to the N₂ mobile phase for another trial. As needed, the water stationary phase was occasionally rehydrated between injections to stabilize the system by flowing 100 µL/min of water through the column for 10 s and re-equilibrating with N₂.

In some experiments, the pH of the water stationary phase was examined *in-situ* by doping it with a small amount of bromocresol green indicator. For this, the inside of a 1 m piece of 250 µm i.d. fused silica capillary tubing was coated with the aqueous indicator solution and placed between the injector and the column. Under magnification, through a small viewing window in the capillary tubing (made by burning off the polyimide coating), digital photographs and videos of the water stationary phase were collected. The indicator colour observed with different mobile phase conditions was used to convey the pH of the water stationary phase inside of the column.

Reagents and Supplies

High purity N₂ and Coleman grade CO₂ (99.99%; equipped with a siphon tube) were used for the mobile phase, while high purity H₂ and medical grade air supported the FID flame (Praxair, Calgary, AB, CAN). Degassed HPLC grade water (Honeywell Burdick & Jackson, Muskegon, MI, USA) was used for system hydration and stationary phase formation. Chemicals for the samples examined here include hexane, acetic acid, propanoic acid, hexanoic acid, cyclohexanecarboxylic acid, sodium benzoate, ethanol, 1-butanol, 1-pentanol (98%; Sigma-Aldrich, Oakville, ON, CAN), and HPLC grade methanol and 1-propanol (99%; EMD Mississauga, ON, CAN). Unless stated otherwise in the text, samples were normally prepared in water to a concentration of about 5-7 µg/µL.

The system was also used to analyze several different samples including vinegar, red wine, mouthwash, aftershave, automotive fuel, and oil sands process water. For these, most samples were purchased from local vendors, while the process water was obtained from a research group on campus and filtered prior to use, and a naphthenic acid mixture (technical grade) was obtained from Sigma-Aldrich. Vinegar was analyzed as received, and the other samples were spiked with target analytes to a concentration of 5-7 µg/µL, except for the fuel/process water samples which were normally prepared at 20-100 µg/µL. All other details are given in the text.

RESULTS

General Operating Characteristics

Initial efforts were made to verify if the system could control the elution of organic acids. For this, various carboxylic acid standards were explored using N₂ and/or CO₂ as the mobile phase with the water stationary phase. As anticipated, it was found that when using N₂, the elution of

carboxylic acids could not be observed under any condition tested, even after 1 h of monitoring. However, when using CO₂, they could be readily eluted from the column. When manipulating the two then, it was indeed found that the elution of such analytes from the column could be externally controlled. Figure 2 shows an example of this with chromatograms of hexanoic acid in the system. For instance, although hexanoic acid could not be eluted using N₂, Figure 2A demonstrates that it could be readily eluted using CO₂, as a prominent peak for this analyte appears within the first 10 min. Conversely, when beginning with a N₂ mobile phase (Figure 2B), no analyte peak is seen during this same period. Rather, it only appears after the switch to a CO₂ mobile phase is made 10 min in, where it has a retention time near 13 min and yields a retention factor of around 5.3 (based on the time after switching).

One feature that was often observed in these experiments was a minor baseline disruption that accompanied the process of switching from N₂ to CO₂. This can be seen in Figure 2B underneath the arrow that indicates when the switch occurred. This was due to a slight system perturbation caused by toggling from the lower (i.e., 9.5 atm) N₂ pressure to the higher (i.e., 60 atm) CO₂ pressure. While a variety of different pressures were examined, it was consistently found that the higher CO₂ pressure range (i.e., 60 atm or more) frequently provided the smoothest baseline, the most stable water stationary phase, and the least system noise during such switching procedures. Higher pressures of N₂ beyond 9.5 atm were not explored. Therefore, given its superior performance, this higher pressure of CO₂ was used throughout these experiments.

In terms of phase characteristics, it should be noted that the water coating on this column has been shown to reproducibly (3% RSD) establish a thickness of about 4 μm (19). As a result, the column offers a relatively large sample capacity near 50 μg of analyte and provides a stable

phase with consistent retention properties (19). Accordingly, analyte retention was also found to be quite reproducible here. For example, the hexanoic acid retention time in Figure 2B yielded a run to run RSD value of 1% and a day to day value of 1.5% (both $n=3$) and the system provided stable operation over several months of experiments. Further, the carboxylic acids produced the same retention behavior whether they were injected from an aqueous or an organic solvent. Therefore, the elution of such acids from the water stationary phase can be directly and reproducibly manipulated through alternating between N_2 and CO_2 during separations.

Given that the elution of such acids could be controlled in this way, it was of further interest to investigate the effect of the time at which the switch to CO_2 was made. For this, hexanoic acid was injected into the system at $80^\circ C$ and the mobile phase was switched from N_2 to CO_2 after increasing periods of time. Figure 3 displays the results, which show that the analyte peak can be held on the column for relatively long periods of time and then readily eluted in each case after the switch to CO_2 is made. For instance, the peak can be held on the column for up to nearly 1 h before being eluted, with little change in its appearance. However, as can be seen, after 1 h or more, the hexanoic acid peak starts to erode and broaden from being held on the column for such a length of time. This effect was also temperature dependent. For example, at $100^\circ C$, the effects of peak broadening and erosion were more apparent after about 35 min. None the less, this demonstrates that the retention of such analytes in this system can also be directly controlled in time over reasonably long periods.

It is also interesting to note that column length had no apparent impact on the ability to retain such acids before subsequently eluting them with CO_2 . For instance, under the same conditions of a $100^\circ C$ oven temperature and switching from a N_2 to CO_2 mobile phase after 10 min, hexanoic acid did not elute from either a 10 m or a 2 m column until after the change was made.

However, as anticipated, other neutral compounds (e.g. alcohols, alkanes, etc.) continued to elute from either column (using either mobile phase) but their retention was proportionately reduced with the shorter length. In this way then, by selectively controlling the elution of such organic acids, the resolution achieved between them and other neutral components in sample mixtures can be directly manipulated relatively independent of column length. This can be beneficial in helping to manage interfering or co-eluting compounds in chromatographic analyses.

Figure 4 illustrates this with the separation of a mixture of standard alcohols containing some carboxylic acids. As seen, Figure 4A shows that when the mixture is separated in a conventional mode using only a CO₂ mobile phase, the alcohols and acids separate in a normal phase fashion with the more polar analytes being retained longer than the less polar ones. This is the same behavior that has been noted previously for the water stationary phase (19, 20). However, also in this trial, hexanoic acid and methanol almost entirely co-elute and are unresolved from one another, making their quantification challenging. Alternatively, Figure 4B demonstrates that when N₂ is initially used as the mobile phase, the neutral alcohols can again be readily separated in a similar fashion on the water stationary phase. However, the acids do not elute until the mobile phase is switched to CO₂. As a result, hexanoic acid and methanol no longer co-elute and they are greatly resolved from each other. Thus, by controlling the elution behavior of such analytes, the water stationary phase system can provide unique and beneficial selectivity in separations.

Separation Mechanism

Since the underlying premise for controlling the analyte elution behavior above is the ability to acidify the water stationary phase when moving from a N₂ to a CO₂ mobile phase, it was of interest to further explore this aspect of the system. As such, experiments were conducted to

monitor the pH of the water stationary phase *in-situ*. For this, a small fused silica capillary shunt was added to the column inlet and a portion of the polyimide coating was removed so that a window was created to view the stationary phase inside of the capillary. The column (including the shunt) was then coated as usual with the water stationary phase, which was also laced with a small amount of the pH indicator bromocresol green. This indicator was chosen because its colour change is well characterized over the neutral to acidic pH range of interest. For instance, it is yellow below a pH of 3.8, blue above a pH of 5.4, and green at intermediate values. Therefore, by using a microscope to image the stationary phase along the fused silica capillary wall, a better understanding of the water stationary phase pH under different conditions could be obtained.

It was found that the indicator within the water stationary phase clearly displayed starkly different colors when in contact with either mobile phase. For example, Figure 5 displays typical examples of the images obtained in these trials. As seen, Figure 5A shows that when a N₂ mobile phase is present, the indicator is a prominent blue color, signifying that the pH of the stationary phase is above 5.4. Conversely, Figure 5B demonstrates that when a CO₂ mobile phase is present, the indicator is distinctly yellow in color, signifying that the stationary phase pH is below 3.8. Therefore, the acidity of the water stationary phase can indeed be controlled externally by altering the mobile phase used. This in turn can then be used to control the retention behavior of certain analytes. For example, since the pK_a of the alkyl carboxylic acids above is about 4.8, it follows that they would be largely ionized and heavily partitioned in the water stationary phase when N₂ is present. However, in the presence of CO₂, the same acids would be protonated/neutral and could therefore be readily eluted under these conditions.

With respect to system dynamics, this change in pH was also found to be quite rapid. For example, the transformation in color from blue to yellow upon switching from N₂ to CO₂ was

visually observed to be practically instantaneous. Of note, measurement of this process indicated that the pH of the water stationary phase completely changed from above 5.4 to below 3.8 in only 2 s. Likewise, after releasing excess CO₂ pressure from the system, this was also true for the switch back to N₂. As such, there is no major delay in the water stationary phase responding in pH terms to the change in mobile phase, which facilitates direct control of elution.

Another interesting observation made during the above experiments was that the pH of the water stationary phase also appeared to readily change as a function of CO₂ pressure in the system. For example, without N₂ present, as the CO₂ pressure alone was decreased from 60 atm towards ambient conditions, the color of the pH indicator could be clearly seen to turn from yellow to green, signaling that the pH had turned from below 3.8 to near 4.8. Therefore, the system CO₂ pressure has some capacity to also independently control the pH of the water stationary phase. This is reasonable since the water phase pH is directly dependent on the amount of CO₂ dissolved, which in turn is well known to correlate with the applied CO₂ pressure (23). Accordingly then, at lower CO₂ pressures, the resulting decrease in solubility can directly impact the pH of the water stationary phase.

Given this, it was interesting to see if this variable could also be used to manipulate analyte retention in a fashion similar to the above mixed mobile phase trials. To explore this, some experiments were carried out isothermally at various pressures of CO₂ (i.e., using a standard gas cylinder and regulator) to determine its effects on analyte retention. Under certain conditions it was found that analyte elution could in fact also be controlled by altering CO₂ pressure alone. For example, using a shorter 2 m column to reduce analysis times and an oven temperature of 100 °C, it was found that altering the CO₂ pressure below about 10 atm could directly impact analyte elution. Figure 6 demonstrates the typical results obtained for hexanoic acid. As seen in

Figure 6A, when using a CO₂ pressure of 9.5 atm, hexanoic acid still readily elutes from the water stationary phase. Conversely, in Figure 6B, it can be seen that when the CO₂ pressure is dropped to 5.5 atm, no peak is eluted even after 50 min of monitoring. Rather, a somewhat noisy baseline is only observed at these lower pressures.

It should be noted that the CO₂ density is very low at these pressures (≤ 0.014 g/mL) and, therefore, the sizable loss in analyte retention cannot be ascribed to mobile phase solvating power effects since it is negligible under these conditions. Likewise, the spikes in Figure 6B were also not found to be from restrictor plugging issues. In fact, it was found that hexanoic acid would continue to elute down to pressures of about 6.8 atm, but below this it suddenly would not. Of note, only a slight change in pressure (about 20 psi) at this level appears to be sufficient enough to change the stationary phase pH such that elution can be controlled.

Incidentally, since temperature can also directly impact the aqueous solubility of CO₂ (23), this parameter was similarly explored for its ability to control analyte elution with the water stationary phase. Again, using the same 2 m column as above and employing a CO₂ mobile phase pressure of 6.8 atm, it was found that only moderate temperature changes were enough to alter elution behavior. Figure 7 demonstrates this for hexanoic acid injections at different temperatures. As seen in Figure 7A, at 180°C, the peak readily elutes from the water stationary phase. However, when the system temperature is increased to 200°C (Figure 7B), no peak is observed even after nearly 25 min and increased baseline noise and periodic spiking similar to Figure 6B appear instead. Then, when the temperature is subsequently reduced again to a lower setting (Figure 7C), the system recovers and the peak readily appears as before. Thus, by altering the aqueous solubility of CO₂ through adjusting column temperature, it seems that the water stationary phase pH (and hence analyte elution) can also be controlled in this manner.

Overall then, it appears that both the pressure and temperature of a CO₂ mobile phase alone can also be used to control analyte elution in a similar manner to the earlier trials that invoke switching between N₂ and CO₂. This is likely a direct result of altering the CO₂ solubility in the water stationary phase, and hence its pH. However, as alluded to earlier, it was found that the system was difficult to operate at the lower CO₂ pressures necessary for this, and greatly increased baseline noise was often observed. As well, various efforts to remedy this issue were not successful. Thus, while more optimization of such an approach may prove useful in the future, continued investigations of this nature were not pursued further here. As such, the alternating N₂ and CO₂ mobile phase system detailed above was deemed the best suited for this.

DISCUSSION

Applications

The above findings suggest that this pH tunable water stationary phase system may potentially be able to provide useful selectivity in analytical separations. In order to further explore this, several different samples containing target organic acids were investigated with it. As well, since the water stationary phase can be readily replenished and possesses a relatively large sample capacity (19,20), each sample was directly injected in a neat, undiluted fashion in attempts to probe the system capabilities in this regard and potentially simplify the analyses.

Initially, some relatively simple samples were explored. The first of these were several different types of vinegar that were analyzed for acetic acid, one of the major components present. In all cases it was found that the acetic acid peak was readily observed only after switching from a N₂ to a CO₂ mobile phase. For example, Figure 8A shows the results obtained for an injection of apple cider vinegar. As seen, a small number of minor sample components are

observed during elution with N_2 . However, upon changing to CO_2 after 10 min, the dominant acetic acid peak appears around 14 min, along with a minor unknown peak near 11.5 min. Conversely, Figure 8B illustrates an analysis of a spoiled red wine sample. In this instance, the dominant ethanol peak appears initially during elution with N_2 , and then once completed, the system is switched to CO_2 after 10 min and the acetic acid impurity can be readily isolated for measurement. This approach also works for other acids as well. For instance, Figure 8C demonstrates a similar analysis of a commercial mouthwash. Again, the dominant ethanol peak is observed early on during elution with N_2 . Then, after changing to CO_2 10 min later, the minor amount of sodium benzoate present (often added as a preservative to dental products (24)) is observed around 17 min as benzoic acid. Similar results were also obtained when analyzing aftershave lotion for sodium benzoate. Thus, such acids and their carboxylate salts can potentially be readily separated and analyzed using this approach. This is useful since, for example, conventional GC methods often require lengthy derivatization of such acids prior to analysis (25, 26), and they are not compatible with direct aqueous sample injections (27). As such, this direct analytical approach could potentially simplify certain analyses.

Of further interest though, through tuning the water stationary phase pH and controlling the release of target analytes, the system presented here could also potentially facilitate the analysis of more complex samples. Figure 9 presents an example of this with the analysis of naphthenic acids. These acids are found in many petroleum sources such as oil sands bitumen, fuels, and wastewater streams from petroleum refining (28-30). Given their environmental toxicity and corrosive nature towards industrial processing equipment, there exists a great amount of interest in monitoring naphthenic acids. Analytically, this often proves to be a significant challenge as these acids are so complex in variety that they are normally determined as a co-eluting 'hump' in

chromatographic separations (28-30). Further though, they also frequently require derivatization in order to be detected and/or eluted by conventional HPLC or GC methods. Even more, since petroleum matrices are so complex, these acids very often cannot be separated from other components in the mixtures, making their determination difficult. As such, naphthenic acids were of interest to examine here with the tunable water stationary phase.

Since naphthenic acids are often monitored in Oil Sands Process Water (OSPW) in efforts to prevent their release into the environment (30), such samples were first investigated here. Figure 9A shows the typical results obtained for a standard filtered alkaline OSPW sample. As seen, the OSPW acquired was relatively clean with little background interference from other matrix components. For instance, once elution with N_2 did not produce any peaks in the first 10 min, the mobile phase was switched to CO_2 . Upon doing so, a large broad peak containing the naphthenic acids then eluted from the column for measurement, as is commonly invoked in conventional methods (28-30). Thus, naphthenic acids can be analyzed by this approach. Further, they were determined here from neat injections of the OSPW sample without any analyte derivatization necessary. As such, this can greatly facilitate the analysis of such samples. Incidentally, similar positive results were also obtained from analyzing hexane extracts of the OSPW as well.

However, of even greater interest is the potential of the tunable stationary phase to analyze for these compounds in more challenging complex mixtures. For instance, Figure 9B shows a conventional GC separation of a fuel sample containing naphthenic acids and demonstrates the difficulty in analyzing such samples. As seen, a very complex chromatogram is produced, which displays hundreds of overlapping peaks across this 30 min separation. Further, comparisons with standards of cyclohexanecarboxylic acid and a naphthenic acid mixture indicated that they co-eluted with the other gasoline components from the 10 min mark until the end of the run. These

findings are similar to those normally found for the analysis of naphthenic acids in complex mixtures (28-30) and clearly show that it can be problematic to analyze such samples.

In contrast to this, Figure 9C shows the same sample as analyzed using the tunable water stationary phase system. As seen, two major features are observed in the chromatogram. The first is the bulk gasoline matrix, which largely elutes rapidly in the first part of the chromatogram when using a N₂ mobile phase. This has been observed previously (21) and is due to the low aqueous solubility of most of these hydrocarbons, rendering them practically unretained on the water stationary phase. However, after the dominant matrix components present were eluted from the column, the pH of the stationary phase was acidified by switching to a CO₂ mobile phase at 30 min. As seen, this then allows the naphthenic acids to readily elute from the column as a broad collective peak. Therefore, by invoking the tunable water stationary phase, the analysis of samples such as the one shown in Figure 9 can be greatly simplified through both directly injecting them and delaying the naphthenic acid elution until after the majority of dominant interfering matrix components have come off of the column.

CONCLUSIONS

A novel method for controlling the pH of a water stationary phase was demonstrated. Through toggling between a N₂ and a CO₂ mobile phase, the water stationary phase was shown to become respectively less and more acidic. This in turn was used to control the ionization of organic acids on the column, and hence their time of elution. The control over this elution was also found to be relatively independent of time and column length. A similar effect could also be achieved by altering the aqueous solubility of a CO₂ mobile phase alone through changing the system temperature and/or pressure. However, it was deemed unsuitable and was found to

provide less favorable system operating characteristics of noise and instability relative to the N_2/CO_2 switching system. Using the latter system, it was found that by tuning the pH of the water stationary phase, organic acids present in a variety of different samples could be eluted on demand with high selectivity. In terms of efficiency, it should be noted that while the 10 m column used here provided somewhat broad but adequate peaks for the present purposes, greater efficiency should be anticipated when using longer columns. Therefore, the results indicate that this approach could potentially simplify the analysis of such acids, and perhaps others as well, in complex samples.

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FIGURE CAPTIONS

- Figure 1:** Schematic diagram of the pH tunable water stationary phase system.
- Figure 2:** Elution profile of an aqueous hexanoic acid test analyte using A) only CO₂ as the mobile phase, and B) N₂ as the mobile phase for the first 10 min then switching to CO₂ (indicated by the arrow). N₂ is 9.5 atm and CO₂ is 60 atm. Oven temperature is 100°C.
- Figure 3:** Elution of a hexanoic acid test analyte after switching from a N₂ to CO₂ mobile phase after A) 10, B) 20, C) 30, D) 40, E) 50, F) 60, and G) 90 min. Oven temperature is 80°C. Other conditions as in Figure 2.
- Figure 4:** Separation of an analyte mixture in A) a conventional mode using only a CO₂ mobile phase and B) the current system using N₂ as the mobile phase and switching to CO₂ after 35 min. Oven temperature is 100°C. Other conditions as in Figure 2. Analytes are: 1) 1-pentanol, 2) 1-butanol, 3) 1-propanol, 4) ethanol, 5) methanol, 6) hexanoic acid, and 7) propanoic acid.
- Figure 5:** Images of the water stationary phase containing bromocresol green pH indicator in the presence of A) a N₂, and B) a CO₂ mobile phase.
- Figure 6:** Chromatograms for hexanoic acid injections using only a CO₂ mobile phase at A) 9.5 atm, and B) 5.5 atm. The oven temperature is 100°C. Column length is 2 m.
- Figure 7:** Chromatograms for hexanoic acid injections at an oven temperature of A) 180°C, B) 200°C, and then C) 170°C using 6.8 atm of CO₂ as the mobile phase. Column length is 2 m.
- Figure 8:** Chromatograms for neat injections of A) apple cider vinegar, B) red wine containing acetic acid, and C) mouthwash containing sodium benzoate. The oven temperature is 120°C. The mobile phase is switched from N₂ to CO₂ after 10 min. Other conditions as in Figure 2.
- Figure 9:** Chromatograms for neat injections of oil sands process water (A) and fuel (B, C) samples containing naphthenic acids using the tunable water stationary phase SFC system (A, C) and conventional GC (B). For A and C the oven temperature is 100°C. The mobile phase is switched from N₂ to CO₂ after 10 min (A) and 30 min (C). Other conditions as in Figure 2. For B, a 30 m x 0.25mm i.d. DB-5 column was used with 1.6 mL/min of N₂ carrier gas. The split injection ratio was 1:50. A temperature program of 60°C for 5 min, then 10°C/min to 200°C was used. The injector and detector were maintained at 300 °C.

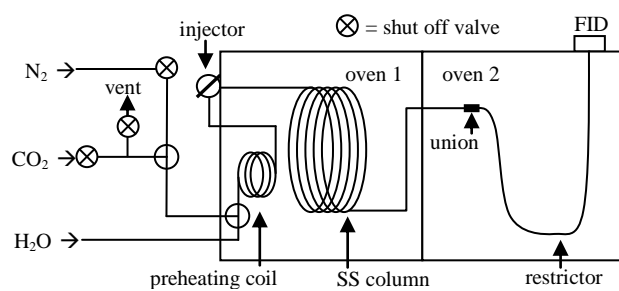


Figure 1

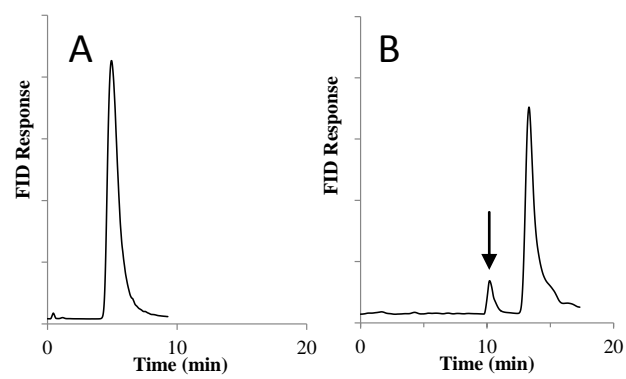


Figure 2

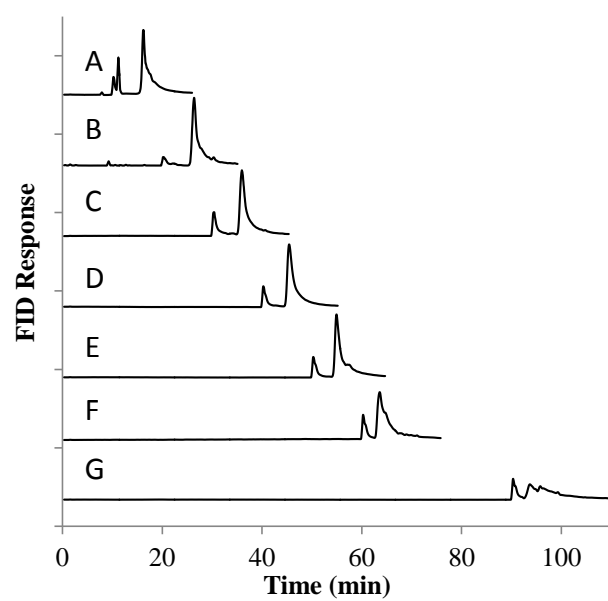


Figure 3

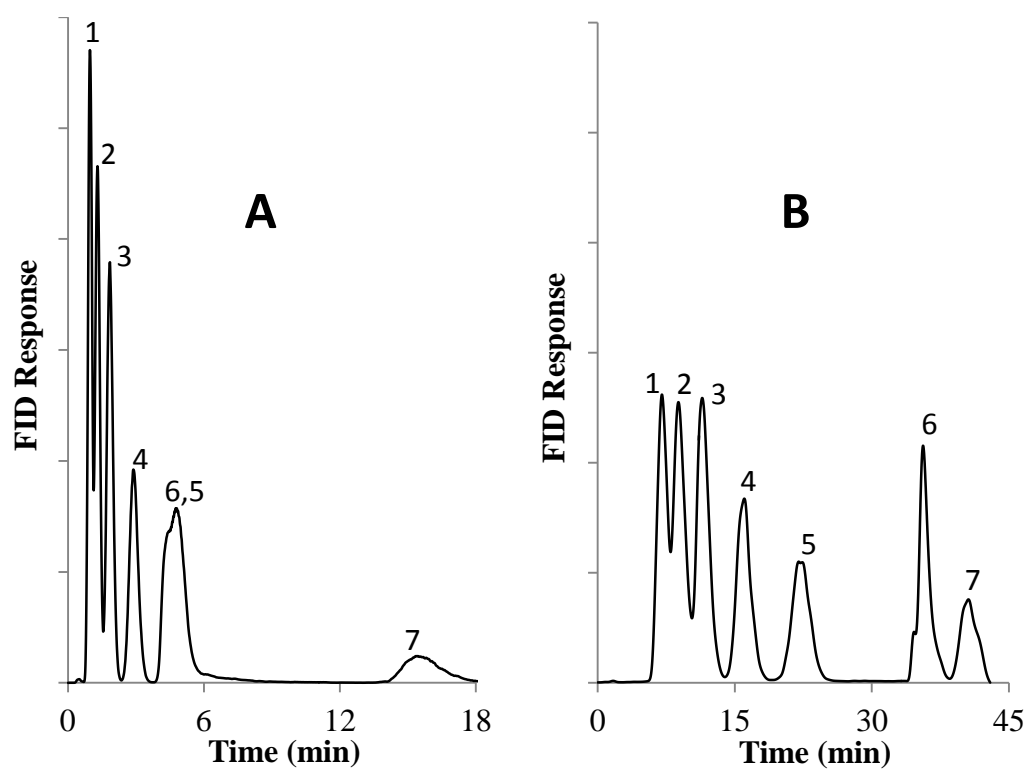


Figure 4

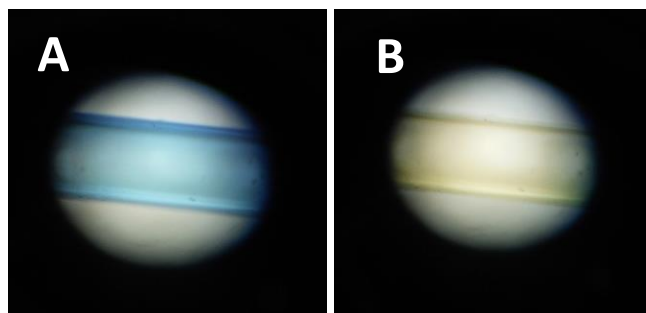


Figure 5

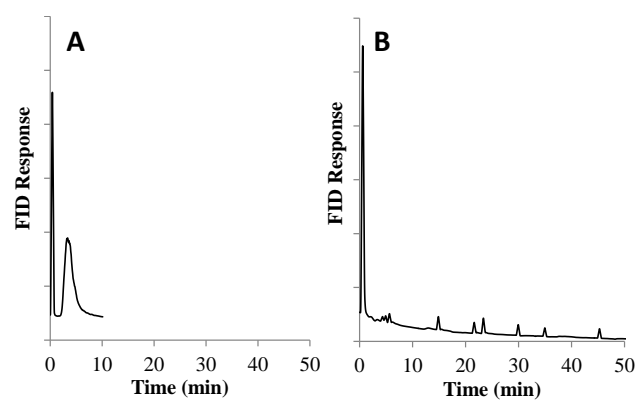


Figure 6

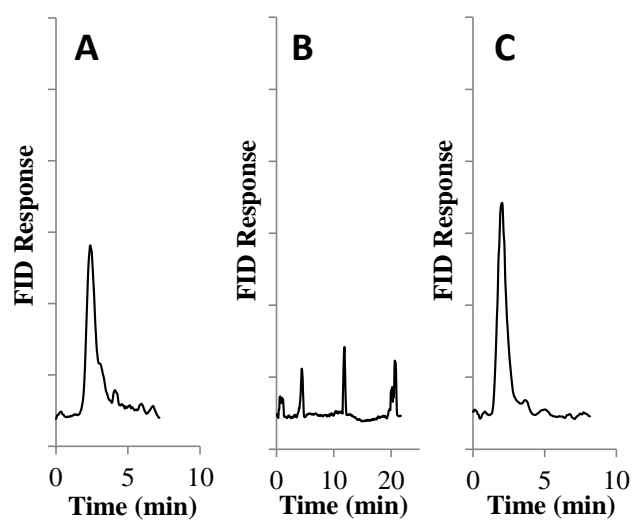


Figure 7

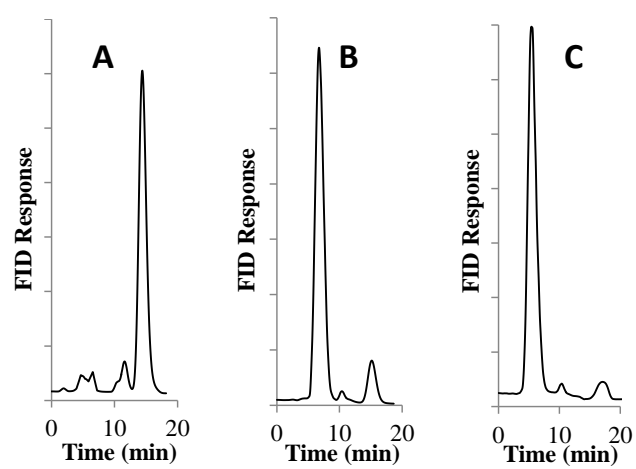


Figure 8

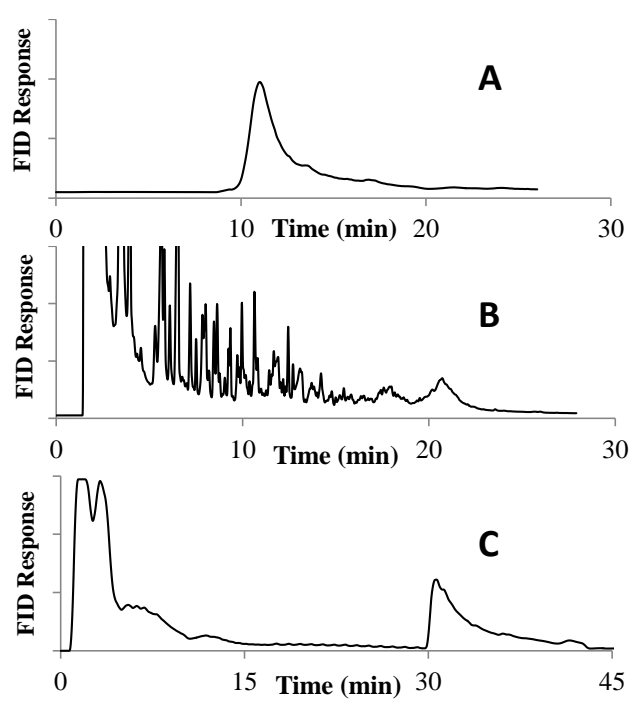


Figure 9