

Examining the Quenching Effects of Modifiers in Packed Column Supercritical Fluid Chromatography with Flame Photometric Detection

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Résumé

Nous avons examiné les effets inhibiteurs de modificateurs organiques dans des colonnes remplies de chromatographie supercritique (pSFC), avec détection par photométrie de flamme (FPD). Les expériences FPD précédentes sur colonne capillaire SFC montrent que des concentrations relativement grandes de modificateurs organiques ajoutés (i.e. 20%) ne causent pas d'inhibition sévère de l'émission. Cependant, en raison des débits de colonne importants, l'inhibition est plus marquée en pSFC. Nous avons observé que de très faibles quantités (près de 0.5%) de modificateur ajouté au dioxyde de carbone supercritique inhibait complètement les émissions chimiluminescentes du soufre et du phosphore. Ce phénomène a été observé de façon consistante pour plusieurs modificateurs communs ajoutés à la phase mobile à des concentrations variables. Les résultats montrent que l'augmentation du contenu en carbone du modificateur cause une augmentation de l'effet inhibiteur. D'autres mesures suggèrent qu'un modificateur avec état d'oxydation plus élevé amène moins d'inhibition. Les résultats indiquent que FPD n'est pas une méthode de détection convenable pour les applications pSFC qui requièrent des phases mobiles typiques modifiées.

Abstract

The quenching effects of organic modifiers are examined in packed-column supercritical fluid chromatography (pSFC) with flame photometric detection (FPD). Previous FPD experiments with capillary-column SFC demonstrate that relatively large percentages of added organic modifiers (i.e. 20%) do not quench emissions severely. However, due to the larger column flows used, quenching is found to have a much greater effect in pSFC. It was observed that very low amounts (near 0.5%) of modifier added to supercritical carbon dioxide completely quenched the chemiluminescent emissions of sulfur and phosphorus test analytes. This was consistently observed for several commonly used modifiers added to the mobile phase at varying concentrations. Results show that, as the carbon content of the modifier increases, the quenching effect also increases. Further findings suggest that a modifier with a higher oxidation state produces less quenching. Results indicate that the FPD is not a practically suitable detection method for pSFC applications requiring typical modified mobile phases.

Keywords: supercritical fluid chromatography, flame photometric detection, organic modifiers, quenching, carbon dioxide.

Introduction

Supercritical fluid chromatography (SFC) provides a complimentary method to both high performance liquid

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chromatography (HPLC) and gas chromatography (GC) (1). As such, it can perform HPLC-like separations while using GC detection methods (2). A popular GC detection method used in this area is the flame photometric detector (FPD), which yields a selective and sensitive response toward compounds containing sulfur and phosphorus. It consists of a cool hydrogen-rich flame that reduces and excites analyte molecules to chemiluminesce. For sulfur and phosphorus, the respective chemiluminescent emission produced is S_2^* near 394 nm and HPO^* near 526 nm (3).

A major problem that arises in GC-FPD is the quenching of analyte emission that occurs due to co-eluting hydrocarbons also present in the detector (3,4). The mechanism of this quenching effect is not fully understood, but several possibilities have been proposed (4-6). For example, it has been suggested that hydrocarbon fragments scavenge vital flame species, such as hydrogen radicals, which are needed for chemiluminescent excitation (4). Early on, Rupprecht and Phillips noted that more oxidized molecules such as carbon dioxide produced minimal quenching (7), which bodes well for SFC-FPD since carbon dioxide is the most common mobile phase.

To date, the FPD has been primarily used in capillary-column SFC (cSFC), which employs very low column flows in the microliter per minute range. Using carbon dioxide, a blue background emission is generated in the FPD, which can cause a significant baseline offset (8). Though this does not greatly hinder the cSFC-FPD detection limits for sulfur (8,9), the milliliter per minute range of column flows used in packed column SFC (pSFC) seriously amplifies this problem and deteriorates detection limits (10). In the latter case, carbon dioxide creates a background emission five orders of magnitude larger than an argon mobile phase, which does not emit in the FPD (10).

Similar to GC-FPD, co-eluting hydrocarbons quench emission in cSFC-FPD when their concentrations are much greater than the analyte (8,9). Unlike the inert carrier gases used in GC, however, the mobile phases in SFC can be considerably different in character. For instance, since supercritical carbon dioxide ($SC-CO_2$) is non-polar, organic solvents are often added to it to increase the polarity of the mobile phase for eluting more polar analytes (11). The continuous flow of carbon that this presents to the detector in SFC-FPD is significant. Despite this, little information exists regarding the extent

of quenching of FPD response by mobile phase modifiers in SFC. In this regard, it has been reported that quenching is not expected to greatly interfere when using up to 20% of methanol as a modifier in cSFC-FPD (12).

Quenching in the FPD has been examined for the organic mobile phases used in HPLC, although the results vary. For example, McGuffin and Novotny reported a slight enhancement in phosphorus emission when using 10-25% of aqueous alcohol or acetone solutions as the mobile phase (13,14). By contrast, Kirkland *et al.* reported a 50% reduction in phosphorus emission when using only a 1% aqueous methanol solvent and 100% quenching when using pure organic solvents (15). However, the former study used microcapillary LC column flow rates of less than 5 μ L/min, whereas the latter used conventional LC column flows of 2.4 mL/min. This is very significant since an increase in the flow of hydrocarbon entering the flame increases quenching (4).

pSFC is by far the most dominant mode of SFC in use today (2). Since it uses much higher flow rates than cSFC, this should have a significant impact on the affect of modifiers in pSFC-FPD. However, no studies have characterized the FPD emission quenching produced by various common modifiers under typical pSFC conditions. Such information would be very beneficial in applications of this method attempting to separate and detect polar compounds by pSFC-FPD. This study examines the extent of quenching that different modifiers have on the emission of sulfur and phosphorus in pSFC-FPD. Some implications on the influence of the modifier's chemical structure are also presented and discussed.

Experimental

The pSFC system used was a Gilson series SF3 (Gilson, Villiers-le-Bel, France) comprised of a dual reciprocating pump solvent delivery system (model 308 and 306) set to 1.0 mL/min, a 20 μ L Rheodyne injector (model 7725i), a column oven (model 831) set to 40°C, and a back pressure regulator (model 821) set to 15 MPa. Mobile phase delivery was facilitated by continuous cooling of the pump heads with chilled water (-5 to 5°C) from a refrigerated circulating bath. A 250 mm x 4.6 mm Econosil CN column (Alltech, Deerfield, IL, U.S.A.) was used for separations. The detection system used was a Shimadzu model GC-8A (Shimadzu, Kyoto, Japan) GC-FPD system. The FPD is connected to the pSFC instrument downstream from the back pressure regulator

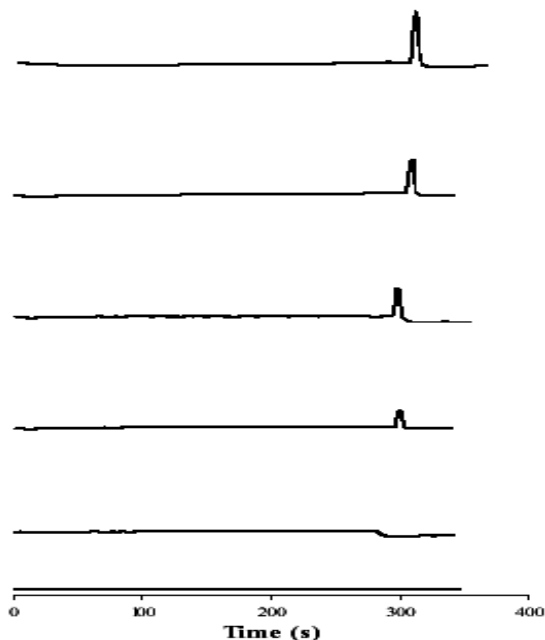


Figure 1. pSFC-FPD chromatograms of tetrahydrothiophene produced with (from top to bottom) 0%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% methanol added to the CO₂ mobile phase.

by a 4 foot length of 1/16" x 0.01" i.d. stainless steel tubing (Alltech). This is used to guide the effluent from the pSFC into the GC oven through a side port. Using a stainless steel zero dead volume union (Alltech), this tubing is connected to a 20 cm length of 0.1 mm i.d. deactivated fused silica restrictor tubing (Alltech), which extends into the base of the FPD burner approximately 4 cm below the flame. The pSFC effluent combines with the hydrogen and flows concentrically around the central tube, delivering air to the flame.

Typical FPD flame-gas flow rates used were 300 mL/min of hydrogen and 90 mL/min of air. The base of the FPD detector was maintained at a temperature of 270°C. Flame emissions were monitored using an R-1104 photomultiplier tube (PMT) (wavelength range of 185-850 nm; Hamamatsu, Bridgewater, NJ, U.S.A.). Interference filters were used to selectively monitor the emission of sulfur (393 nm interference filter; 11 nm bandwidth) and phosphorous (520 nm interference filter; 9 nm bandwidth).

Instrument-grade carbon dioxide and high purity hydrogen and air were obtained from Praxair (Praxair, Calgary, AB, Canada). Tetrahydrothiophene (97%; Fluka Chemika, Oakville, ON, Canada), ethyl sulfide (98%; Aldrich, Oakville, ON, Canada), trimethyl phosphite (99

+ %; Aldrich), and tris(pentafluorophenyl)phosphine (97%; Aldrich) were dissolved in acetone (99.5%; EMD Chemicals, Gibbstown, NJ, U.S.A.) at concentrations of approximately 0.1-1 µg/µL for the sulfur compounds and 10 µg/µL for the phosphorus compounds. The modifiers examined were methanol (99.8%, EMD Chemicals), ethanol (absolute, EMD Chemicals), isopropanol (99.5%; EMD Chemicals), n-butanol (99.8%; Aldrich), 1,4-dioxane (99.0%; BDH Inc., Toronto, ON, Canada), tetrahydrofuran (99.5%; BDH Inc.), acetone (99.5%; EMD Chemicals), and formic acid (88%; BDH Inc.).

Test analytes were repeatedly injected in the presence of various levels of modifier in the pSFC-FPD system and measurements of peak area were made for each. Quenching was determined as the ratio of the detector response (R) in the presence of modifier to that obtained without any modifier present (R₀). Values of R/R₀ were then tabulated for each quantity of modifier investigated. All other variations are described in the text.

Results and Discussion

Initial efforts focused on the quenching effects of methanol, the most commonly used modifier in pSFC. For a typical modifier concentration of 5%, no peak was seen for tetrahydrothiophene. Subsequently, lower percentages of modifiers were tested. At 0.1% addition, a signal was seen, although it was reduced compared to that obtained using pure CO₂. Ultimately, the signal was completely quenched after adding only 0.5% methanol. This effect is demonstrated chromatographically in Figure 1, while Figure 2 presents quantitative values of R/R₀ as a function of methanol addition. As expected, since S₂* emission is produced by all sulfur compounds, the same results are found for other analytes such as ethyl sulfide. For example, the mobile phase concentration of methanol required to quench the respective emissions produced by tetrahydrothiophene and ethyl sulfide to half of their original values agrees within about 0.02%.

Phosphorous, as trimethyl phosphite, was also examined under these conditions, and the results are included in Figure 2. As seen, similar to sulfur, phosphorous emission is also completely quenched when using comparable amounts of methanol modifier. Unlike the sulfur test analytes, however, there is an initial increase in the phosphorus signal with the addition of 0.1 to 0.2% methanol before it is entirely quenched. This is because minor loss of the polar analyte occurred on the column

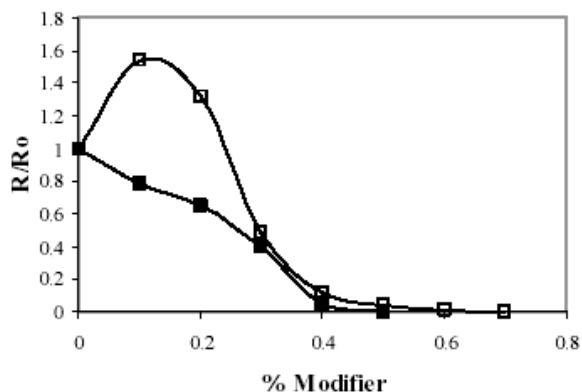


Figure 2. Quenching effects of methanol as a modifier on the peak areas of the sulfur emission of tetrahydrothiophene (■) and phosphorus emission of trimethyl phosphite (□) in pSFC-FPD. The response ratio is with (R) and without (Ro) modifier present. The flow rate of carbon dioxide is 1.0 mL/min at a pressure of 15 MPa.

while establishing the Ro values with pure CO₂. With the subsequent addition of small amounts of modifier, more of the analyte is delivered to the detector, increasing the observed signal despite the minimal quenching that also occurs. Regardless, this effect is short lived, as the signal increasingly diminishes beyond 0.1% modifier. Again, as

with sulfur, other phosphorous compounds, such as tris(pentafluorophenyl)phosphine were examined and displayed similar quenching behavior. Of note, at 0.3% methanol addition the R/Ro values obtained for the phosphorus analytes differed by only 0.05, and both fell to zero for 0.7% methanol addition. Therefore, it appears that considerable quenching occurs in the FPD under typical pSFC conditions even when trivial amounts of methanol are used.

Since increasing the carbon content of the co-eluting hydrocarbon increases quenching in GC-FPD (5,7), other alcohol modifiers with increasing carbon number were examined. The quenching effects of methanol, ethanol, isopropanol, and n-butanol are shown respectively for sulfur and phosphorus emissions in Tables 1 and 2. As seen, for modifiers of increasing carbon number the extent of quenching is indeed increased. Further, this occurs to the same degree for both sulfur and phosphorus emissions. This finding agrees with the GC-FPD results of Aue and Sun, who observed several elements to exhibit similar quenching by equivalent amounts of hydrocarbon. They then interpreted this as hydrocarbons quenching the excited flame rather than the excited analyte (4).

The effect of quenching due to the chemical structure

Table 1. Effects of different modifiers on the emission of sulfur as tetrahydrothiophene in pSFC-FPD

Modifier Addition (%)	(R/Ro) x 100							
	Methanol	Ethanol	Isopropanol	Butanol	THF	Dioxane	Acetone	Formic Acid
0	100	100	100	100	100	100	100	100
0.1	78.5	67.4	16.6	0	0	44.6	42.5	80.7
0.2	64.3	58.3	0			10.8	0	72.7
0.3	39.9	34.5				0		39.9
0.4	4.7	8.6						32.9
0.5	0	0						20.2
0.6								25.5
0.7								18.0
0.8								13.7
0.9								14.7
1.0								15.0
2.0								4.5
3.0								0

Table 2. Effects of different modifiers on the phosphorus chemiluminescence emission of trimethyl phosphite in pSFC-FPD

Modifier Addition (%)	(R/R ₀) x 100							
	Methanol	Ethanol	Isopropanol	Butanol	THF ^a	Dioxane ^a	Acetone ^a	Formic Acid ^a
0	100	100	100	100	100	100	100	100
0.1	153.8	125.1	22.9	0	0	53.9	59.2	114.1
0.2	131.4	78.7	6.5			20.5	20.2	86.8
0.3	18.8	20.4	3.4			0	0	74.0
0.4	11.8	25.9	0					56.8
0.5	4.3	0						14.4
0.6	1.2							12.9
0.7	0							13.1
0.8								12.1
0.9								11.6
1.0								13.7
2.0								7.5
3.0								0

^a experiments done using tris(pentafluorophenyl)phosphine

of the modifier was also briefly explored. Tables 1 and 2 include the findings for some other solvents investigated. For instance, the signals from both sulfur and phosphorus are completely quenched with the addition of just 0.1% THF. Likewise, the same occurs when using n-butanol as a modifier since the two have similar chemical formulas. By comparison, when using 1,4-dioxane as a modifier, complete quenching does not occur until 0.3% addition. Although dioxane has four carbon atoms, like butanol and THF, it is also more oxidized having two oxygen atoms. Thus, in accordance with the findings of Rupprecht and Phillips, relatively less quenching is observed for dioxane.

It is also interesting when dioxane is compared to two molecular fragments of ethanol. For example, Tables 1 and 2 show a considerable difference between the quenching observed for ethanol and dioxane in terms of their concentration in the mobile phase. However, when comparing them with respect to the absolute flow of carbon in the flame, their similarity in structure is more evident. This effect is demonstrated in Figure 3, which compares the relative response against the carbon flow (g C/s) entering the FPD. These values were obtained

for comparison using the density and column flow rate of the modifier. As seen, unlike the tabulated values, the quenching observed is now very similar between the two modifiers. In this way, dioxane might be considered as delivering similar fragments as ethanol to the flame, but at twice the rate. Such an effect is thus compensated for when considering purely carbon flow. While this agrees with others who have examined quencher structure in GC-FPD (16), more modifiers need to be examined before this can be established for pSFC-FPD. However, it is interesting to note that studies have shown that most hydrocarbons are pyrolyzed to similar methyl or methane fragments in the hydrogen-rich zone beneath diffusion flames (17). Thus, if this occurred for modifiers in the FPD, then this may be a reason as to why dioxane and ethanol exhibit these similarities.

Tables 1 and 2 also include comparisons made between acetone and isopropanol. These two modifiers have similar chemical structures and only differ by the second carbon being more oxidized in acetone. Here, it can be seen that about 2.5 times more signal remains for 0.1% acetone than with equivalent isopropanol for both sulfur and phosphorus emissions. While both modifiers

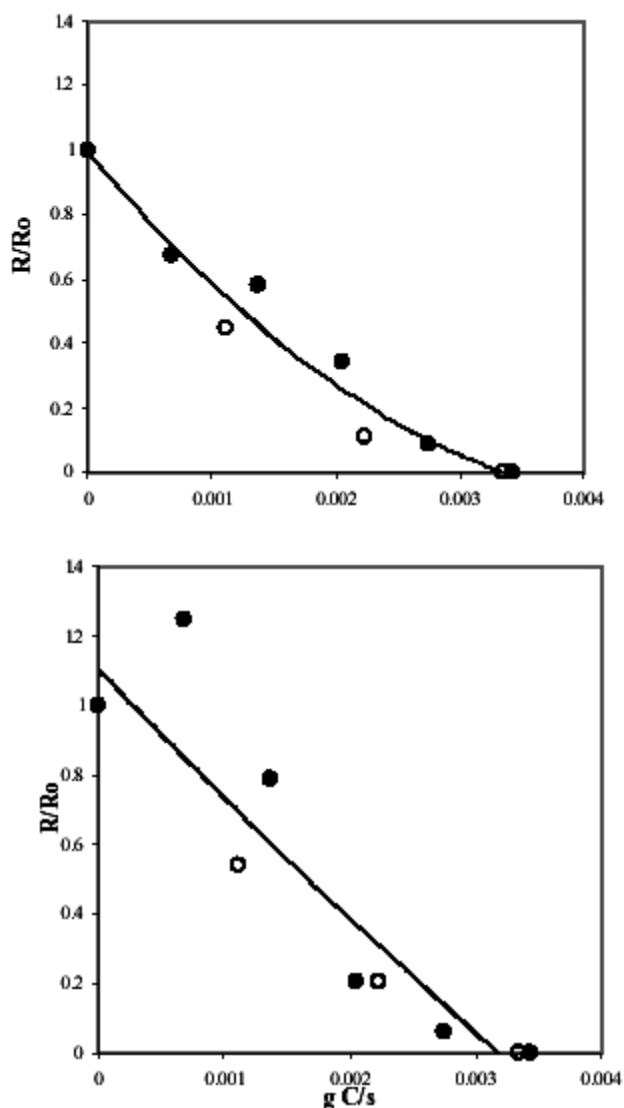


Figure 3. Quenching effects of ethanol (●) and 1,4-dioxane (○) on sulfur emission (top) and phosphorus emission (bottom) as a function of carbon flow (g C/s). Conditions as in Figure 2.

display 100% quenching for 0.2 – 0.3% addition, further intermediate flows could not be examined with the pump.

Formic acid, a one carbon molecule that is highly oxidized, was also investigated. As seen in Tables 1 and 2, the sulfur and phosphorus emissions are noticeably less quenched at higher modifier percentages (e.g. greater than 0.4%) than with methanol or other modifiers. In fact, signal is still measured at amounts of 2% modifier added. Again, similar to the results of Rupprecht and Phillips (7), these findings suggest that modifiers in a greater oxidation state exhibit less quenching. While other investigations of oxidation state were attempted with modifiers such as ethylene glycol and propanediol, they

were too viscous to properly pump through the system.

The severe quenching observed in pSFC-FPD can be rationalized considering that 20% of methanol has been regarded to exhibit little quenching effect for cSFC-FPD at an expanded flow of 5 mL/min (12). For instance, this correlates to a column flow of about 8 μ L/min (assuming a 600 times SC-CO₂ expansion), which corresponds to an absolute methanol flow of 1.6 μ L/min. This is about the same methanol flow achieved for a pSFC flow rate of 1 mL/min using 0.1% modifier addition where little quenching is also observed. Since the FPD flame size is relatively consistent, this is why typically little to no quenching is seen in cSFC, but major quenching is observed in pSFC when using similar concentrations of modifier.

It should be noted that some improvement on quenching may be possible by using dual flame FPD (dFPD). For instance, using cSFC-dFPD, Markides *et al.* report that quenching due to co-eluting organic compounds is not observed (18). Koizumi and Suzuki reported that cSFC-dFPD was compatible with modified SC-CO₂ mobile phases and did not report any interference. However, this was not quantified, and when flow rates exceeded 0.2 mL/min, the flame was extinguished (19). In microcolumn LC with dFPD, aqueous alcohol mobile phases did not cause serious quenching at any concentration, whereas acids and acetone did (20,21). Since pSFC will deliver a much larger modifier flow to the flame, it is currently uncertain to what extent dFPD may be effective for reducing quenching in this method.

Conclusions

Quenching was examined for a variety of typically used modifiers in pSFC. For all of the modifiers tested, complete quenching was observed at percentages less than 0.7%. Formic acid is an exception, as complete quenching is observed between 2.0 and 3.0%. This finding, along with other results, suggests that as the modifier is more chemically oxidized, less quenching is observed, similar to that found for GC-FPD experiments. Also, the flow of carbon stemming from the modifier influences quenching, as longer chain alcohols quench more severely than shorter ones. In general, the onset of quenching occurs at very low percentages for all of the modifiers examined. Such low modifier concentrations in the mobile phase are not always very practical for

pSFC, and therefore quenching should typically be expected to cause considerable interference in modified pSFC-FPD.

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