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Packed column supercritical fluid chromatography–mass spectrometry with particle beam interface aided with particle forming solvent

Paul T. Jedrzejewski, Larry T. Taylor*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Abstract

The particle beam interface aided with particle forming solvent was evaluated for packed column supercritical fluid chromatography–mass spectrometric coupling. Fundamental studies on the effects of various operational parameters were undertaken. Factors influencing aerosol formation (nebulizing capillary I.D., particle solvent type and flow-rate) were evaluated. Pure CO_2 and methanol-modified CO_2 were utilized with equal success. The sensitivity of the system was evaluated in terms of limit of detection (LOD). The electron impact scan and single ion monitoring modes were determined to be 10 and 1 ng for caffeine, respectively. These LODs are 10 to 100 times better than previous reported estimates. Separations of carbamate pesticides and polyaromatic hydrocarbons are shown as examples of the system's utility. Electron impact spectra were artifact-free and comparable to on-line library spectra.

1. Introduction

For solutions to many analytical challenges, mass spectrometric (MS) detection coupled to a chromatographic separation medium enjoys much acceptance. This on-line technique offers selectivity, specificity, universality, and sensitivity in this task. Of the available separation media, supercritical fluid chromatography (SFC) presents distinct properties which have implications for both the separation and interfacing regimes. These unique properties offered by SFC stem from the characteristics of the mobile phase — a supercritical fluid. The SFC mobile phase exhibits low polarity, high diffusivity, low viscosity, and the formation of a gas upon decompression from the restrictor. Of the available SFC geometries, packed column SFC has inherent benefits over that of open tubular SFC. These benefits are accrued in method development and in trace analysis. For method development, packed column SFC allows for a larger selection of stationary phases which permits greater choice in selectivity. Due to higher efficiencies per unit of time, faster analysis times are possible. For trace analysis, packed column SFC offers the advantage of on-column enrichment of analyte(s) due to higher column capacity. Packed column SFC traditionally operates under mild conditions, thus the analysis of thermally labile compounds is readily feasible [1–3].

SFC presents a number of features which allows for easier coupling with MS. Due to its volatility, removal of the mobile phase is easier

* Corresponding author.

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achieved, although the gas load still has to be considered. Also, since SFC is typically performed without non-volatile buffers, problems encountered with the presence of these buffers in LC are not faced in SFC. The attributes of MS detection have been demonstrated by the variety of ionization methods which have been coupled with packed column SFC. Ionization methods of positive chemical ionization (PCI), thermospray (TSP), electrospray (ES), atmospheric pressure ionization (API), and electron impact (EI) have all been shown to be successful in analysis and quantification of various analytes via SFC–MS [4–10]. However, all the listed ionization methods (except EI) are 'soft' ionization methods, thus the information content of the spectra is low. Typically the resulting mass spectra contain as a base peak a protonated ($[M+H]^+$) or cationed (e.g. $[M+NH_4]^+$) analyte and little or no structurally relevant fragmentation. EI ionization yields extensive fragmentation due (in part) to the high electron energies (70 eV) used. EI spectra are generally characterized by either low abundance or the absence of a molecular ion peak. Due to the highly reproducible nature of EI spectra, on-line spectral libraries have been generated to aid in identification of compounds.

The coupling of packed column SFC–MS with EI ionization necessitates the presence of an interface to remove the extensive gas loads created by the decompressing mobile phase. To date two interfaces have been used for packed column SFC–EI-MS: the moving belt interface and the particle beam interface. Games et al. have shown successful coupling via the moving belt interface with applications ranging from steroids and bile acids to antibiotics [9–13]. The sensitivity for scan EI was reported in the low nanograms. This particular interface is not very desirable because of the inherited non-robustness of the introduction method, its mechanical complexity, decomposition of thermally labile analytes, and problems with quantitative transfer of non-volatile analytes [14–17]. None of these difficulties, on the other hand, hampered the PB interface to the same extent since this interface is mechanically simpler to operate and maintain. However, the PB interface does present two important disadvantages: complex optimization and poor mass transfer to the ion source which results in a limited sensitivity. In our previous paper [8], it was shown that packed column SFC–PB-MS is a viable technique for identification and quantification of compounds at analytically significant levels. These results were achieved without the addition of a solvent prior to nebulization as has been used by other workers. Elimination of this solvent, the particle forming solvent (PFS), simplified the operation of the interface a great deal. However, this simplification came as a trade off between sensitivity and concentration of modified CO_2. The dependence of sensitivity on mobile phase composition presents a problem with method development. Method development in SFC is achieved in part by pressure, density, temperature, and/or composition programming. The composition programming can be of two forms: step gradient or continual gradient composition ramps. Thus, the high dependence of sensitivity on composition presents a non-ideal situation which will be addressed in this paper.

The PFS has been employed by other workers. However, these ground-breaking efforts by Edlund and Henion [18] and Browner et al. [19,20] only explored the feasibility of the PB interface for packed column SFC–MS coupling. These studies demonstrated the utility of the interface with separations relevant to pharmaceutical, environmental, and polymer areas of interest. The research reported to date has demonstrated applications with only estimates of sensitivity, thus no detailed quantitative data (calibration graphs, LODs, RSDs) were reported. Furthermore, in the case of Henion's work, these studies were performed on a prototype PB interface not fully optimized for either SFC or LC work. Thus, the detection limit was estimated to be in low micrograms. Browner's studies, performed on a laboratory made PB interface, presented pharmaceutically relevant separations and estimated sensitivity in the 100 to 1000 nanogram range. Also, neither research group has presented a coherent study on the effects of various interfaces, PFS, and chromatographic parameters as they impact the performance of the SFC–

PB-MS system nor have they presented detailed quantification data.

In this paper the performance of the PB interface aided by the particle forming solvent for SFC–PB-MS is presented. Aspects of the interface which affect aerosol generation, droplet desolvation, and transport efficiency were evaluated. The effect of particle forming solvent flow-rate as well as the type and composition of PFS were studied. The effects on sensitivity of mobile phase flow-rate and composition were appraised. The quantitative performance of the system was evaluated under scan, extracted ion, and SIM. The analysis of carbamate pesticides and poly-aromatic hydrocarbons (PAHs) are given as applications.

2. Experimental

2.1. Instrumentation

A Model 5988A MS with a 59980A PB interface (both Hewlett-Packard, Palo Alto, CA, USA) was used. The SFC system was the Model 600 Series (Dionex, Lee Scientific Division, Salt Lake City, UT, USA) (Fig. 1) consisting of a syringe pump, oven, injector, and a data station. The injection loop was 0.5 μl in volume. The packed columns used during this study were Deltabond CN (100 × 1 mm I.D., 5 μm particle) and Hypersil Silica (250 × 1 mm I.D., 5 μm particle) both from Keystone Scientific (Bellefonte, PA, USA). For the mobile phase flow-rate and composition studies, a Model 100D (Isco, Lincoln, NE, USA) syringe pump with a controller was used with an injector (Valco Instruments, Houston, TX, USA). Pressure in these systems was maintained by either a linear (25 μm I.D.) capillary or a tapered restrictor constructed of deactivated fused-silica (SGE, Austin, TX, USA). The restrictor was installed in a fused-silica adaptor and connected at one of the inlets to the tee (Valco Instruments) as shown in Fig. 1. The particle forming solvents were sparged with helium and were delivered with a LC pump (Perkin Elmer, Norwalk, CT, USA). Nebulizing

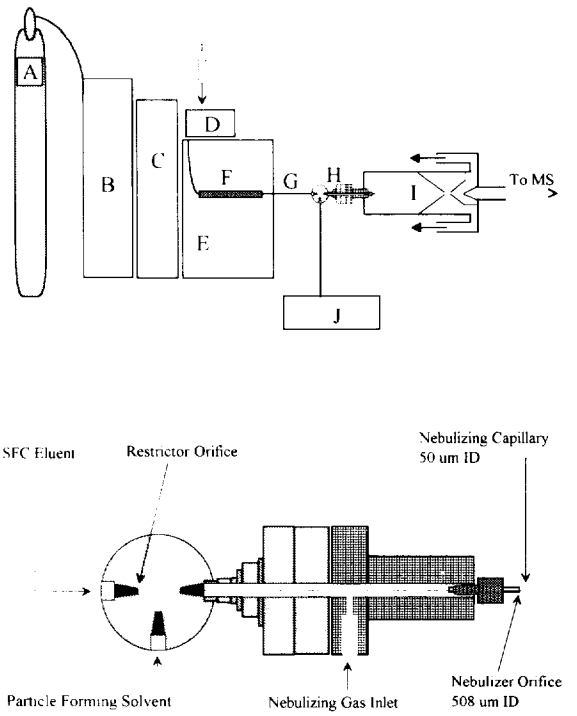

Fig. 1. Top panel shows the configuration of the SFC–PB-MS system: (A) CO_2 cylinder, (B) syringe pump, (C) data station, (D) injector, (E) oven, (F) column, (G) linear restrictor, (H) connecting tee and nebulizer assembly, (I) particle beam interface, (J) particle forming solvent pump. Bottom panel shows detail of the connecting tee and nebulizer assembly.

capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Chemicals

Caffeine (Sigma, St. Louis, MO, USA) was used as received. Pyrene and ammonium acetate were from Aldrich (Milwaukee, WI, USA). Carbamate pesticides and PAHs were obtained from Chem Service (West Chester, PA, USA). Standard solutions were prepared in HPLC grade methanol or acetone (Fisher Scientific, Pittsburgh, PA, USA) and filtered through a 0.2-μm membrane PTFE filter (Supelco, Bellefonte, PA, USA). Methanol-modified CO_2 was obtained from Scott Specialty Gases (Plumsteadville, PA, USA), and SFE/SFC grade CO_2 was obtained from Air Products and Chemicals (Al-

lentown, PA, USA). Solvents used as particle forming solvents were HPLC grade (Fisher Scientific).

3. Results and discussion

The performance of the PB interface with the aid of the PFS was assessed by characterizing the effect of numerous parameters on the quality of information obtained. Although the PB interface has been under scientific evaluation for a number of years in different variations (i.e. Thermabeam, MAGIC, Universal interface), the operation of PB at first principles has not been yet achieved, i.e., rigorous equations defining various characteristics of the PB (e.g. transport efficiency, non-linearity) have not been formulated using basic system variables (e.g. pressure, temperature, etc.). Much of the available data is in qualitative form. However, some areas of the PB have received more attention (i.e. supersonic jet formation, sampling, aerosol formation) therefore we hope to use the available principles to give a more concrete description of the operating mechanism(s). Instrumental parameters (nebulizing capillary I.D., position, nebulizing gas pressure, and desolvation chamber temperature) and PFS parameters (nature, composition, flow-rate) have a direct impact on the aerosol generation in terms of both distribution of the droplets and their size. As a consequence of droplet size and distribution modification, the transmission efficiency of the interface and therefore the sensitivity may be investigated. The focus on chromatographic parameters (mobile phase flow-rate and composition) was for evaluating the dependence (or independence) of the PB on the chromatographic condition required to achieve an efficient separation in SFC. The degree of dependence of PB on SFC has ramification on the utility of this hyphenated technique. Quantitative parameters (LOD, linearity, RSD) were considered in order to assess the application of this system to the analysis of compounds at analytically significant levels. Lastly, the application section demonstrates the utility of the system for the analysis of amenable compounds. The objective was to ensure mass spectra quality and to maintain chromatographic fidelity.

3.1. Instrumental parameters

Internal diameter of the nebulizing capillary

It has been shown by other workers that the internal diameter of the nebulizing orifice dictates the characteristics of the aerosol [21–23]. Thus the modification of this parameter will have an effect on the transport efficiency and consequently on the sensitivity. The PB interface has previously been optimized for liquid flow-rates of 0.4 ml/min with a 100 μm I.D. nebulizing capillary. As will be discussed later, the optimum flow-rate of the particle forming solvent for SFC lies at flow-rates less than 0.07 ml/min. Thus, optimization of the nebulizing capillary internal diameter was evaluated. Three different capillaries were evaluated: 25, 50, and 100 μm I.D. Since all three capillaries were of 370 μm O.D., the gas cross-section area of the gas outlet was constant. For each nebulizing capillary with different internal diameter, two parameters were optimized: its position and the pressure of the nebulizing gas. In order to ensure optimum conditions for each nebulizing capillary, the following optimization algorithm was followed.

1. Optimize nebulizing gas pressure [60 to 15 p.s.i. (1 p.s.i. = 6894.76 Pa) in steps of 5 p.s.i.]
2. Optimize capillary position (+ 10 to −10 in steps of 1)
3. Re-optimize nebulizing gas pressure (previous optimum ± 10 p.s.i. in steps of 2 p.s.i.)
4. Re-optimize capillary position (previous optimum ± 1 in steps of 0.5) (initial conditions: capillary at + 1, nebulizing gas pressure = 25 p.s.i.)

To ensure that the global optimum rather than a local optimum was reached, two iterations of the algorithm were executed. After the conditions were optimized for each capillary I.D., the nebulizing gas pressure and the nebulizing capillary position were evaluated. In the figures that follow each data point is an average of 3

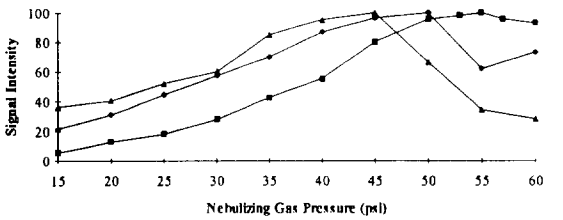

Fig. 2. Effect of nebulizing gas pressure on signal intensity. Conditions: Dionex SFC system; 100% CO_2, 110 atm (1 atm = 101 325 Pa) and 0.25 ml/min liquid flow. FIA of 0.5 μl of 200 ng/μl of caffeine in methanol. PB conditions: nebulizing capillary position 6, −3, −10 for 25 (■), 50 (◇), and 100 μm I.D. (▲) capillaries, desolvation chamber temperature, 40°C. Particle solvent flow-rate, 0.05 ml/min methanol. Other conditions given in Experimental section.

replicates and the RSD values for the data range from 2 to 7.

Optimum nebulizing gas pressures (Fig. 2) were found to be 55, 50, 45 p.s.i. for 25, 50, and 100 μm I.D. nebulizing capillaries, respectively. Since the volumetric flow-rate of PFS was constant, decreasing the capillary internal diameter had the consequence of increasing the velocity of the liquid jet. In order to effectively nebulize these liquid jets the use of correspondingly higher pressures was necessary.

The effect of nebulizing capillary position was evaluated (data not shown). With increasing capillary I.D., the optimum position varied from 6, −3, to −10 (positive values indicate positions outside the nebulizer while negative values indicate those within) for 25, 50, and 100 um I.D. nebulizing capillaries, respectively. After the optimization of the above parameters, the relative sensitivity of the three nebulizing capillaries was assessed. The 100 μm I.D. nebulizing capillary was found to yield the least sensitive results. The 25 μm nebulizing capillary showed an almost two-fold improvement over the 100 μm I.D. capillary. The 50 μm I.D. nebulizing capillary, however, gave the best results: improvement of a factor of 2 over the 25 μm I.D. capillary. For the studies that follow, the 50 μm I.D. nebulizing capillary was used.

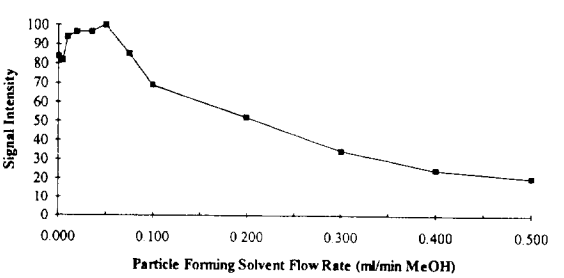

Fig. 3. Effect of particle forming solvent flow-rate on signal intensity. Conditions: Dionex SFC system; 100% CO_2, 110 atm and 0.25 ml/min liquid flow. FIA of 0.5 μl of 200 ng/μl of caffeine in methanol. PB conditions: nebulizing gas pressure, 45 p.s.i.; nebulizing capillary position, −3; desolvation chamber temperature, 40°C. Other conditions given in Experimental section.

Desolvation chamber temperature

The effect of the desolvation chamber temperature was evaluated in the range from 35 to 75°C (data not shown). The optimum desolvation chamber temperature range was quite wide from 35 to 50°C. At temperatures greater than 50°C, a decrease in sensitivity was observed. This decrease was the result of over-desolvation of the droplets. Over-desolvation leads to formation of smaller droplets resulting in lower transport efficiency through the momentum separator and lower sensitivity. Presumably, this decrease in sensitivity is due to a greater fraction of analyte being skimmed off and lost in the pumps. For comparison, under LC conditions with 0.4 ml/min methanol, the optimum temperature has been shown to lie between 28 and 45°C [25,35].

3.2. Particle forming solvent parameters

Particle forming solvent flow-rate

Under LC–MS conditions, the sensitivity of the PB interface is dependent on the mobile phase flow-rate with an optimum of about 0.4 ml/min [24,25]. As alluded to in the preceding section in the discussion of the internal diameter of the nebulizing capillary, the optimum PFS flow-rate for SFC–PB-MS was found to lie at values smaller than 0.4 ml/min. Only after optimizing the above parameters the effect of the PFS flow-rate could be accurately assessed. As shown in Fig. 3, the PFS flow-rate (assessed from 0.001 to 0.500 ml/min) has a profound effect on sensitivity. The optimum sensitivity lies on a

plateau between 0.020 to 0.050 ml/min. The location of the plateau is dictated by adequate aerosol generation with the PFS and effective sweeping of the volume in the connecting plumbing. At flow-rates less than the optimum, conditions exist which foster inadequate aerosol formation due to the lower velocity of PFS liquid jet. Also, at sub-optimum flow-rates, the volume comprising the connecting tubing is inadequately swept leading to dilution of the peak. This band broadening resulted in decreased sensitivity. At flow-rates greater than 0.050 ml/min, the sensitivity rapidly decreased. The severity of this decrease could have been lessened by re-optimizing the conditions (see algorithm above) at each flow-rate. This decrease was visually observed as a scatter of aerosol and a solvent accumulation under the nebulizer. Both of these losses result in a decrease of analyte transport to the detector.

Nature of the particle forming solvent

The pioneering work in the development of the PB interface for LC–MS by Winkler, demonstrated the profound effect of the nature of the LC eluent on the performance of the interface [24]. Winkler evaluated a number of solvents in terms of sensitivity, mean droplet diameter, and particle size distribution. He investigated these effects for hexane, acetonitrile, water, and methanol. In terms of sensitivity, Winkler found that sensitivity improved with decreasing surface tension of the solvent. Thus, in comparison to hexane and methanol, hexane was found to yield more sensitive results. The aerosol of various solvents was characterized by laser Fraunhofer diffraction in order to determine mean droplet diameter and particle size distribution. Winkler found that the mean droplet diameter and particle size distribution positively correlated with surface tension. Thus, the mean droplet diameter and particle size distribution were found to decrease for the following solvents arranged in order of decreasing surface tension: water, acetonitrile, methanol, and hexane. For example, water was found to yield a mean droplet diameter of 11 μm and particle size distribution from 2 to 30 μm. In contrast, an organic solvent such as hexane produced a mean droplet diameter of 6 μm and particle size distribution between 2 and 12 μm.

Recently, the effect of different solvents on sensitivity in LC–PB-MS was further evaluated by Cappiello and Bruner [26]. These workers evaluated the following solvents: water, acetonitrile, methanol, and isopropanol. Confirmation of the findings of Winkler was observed in that sensitivity increased with decreasing surface tension of the solvent. Although this research was performed at liquid flow-rates of only 1 μl/min, the general trend should hold for any flow-rate.

The effect of different PFS on the sensitivity performance of the SFC–PB-MS system was determined with caffeine and pyrene. These two analytes were selected to test for the possibility of solvent–solute interaction. If for the same solvent the two analytes showed significantly different responses, then factors other than solvent surface tension would be responsible for this discrepancy. In Fig. 4 we observed the same trend as reported in the above LC–MS studies, except for toluene and pentane. For both caffeine and pyrene the relative responses increased with decreasing surface tension. Also, the relative responses for both analytes were equivalent within the error of the technique (RSD 2 to 7). Table 1 contains pertinent physical data for the evaluated solvents. However, toluene with a

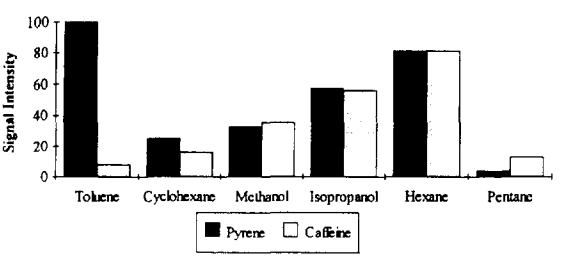

Fig. 4. Effect of various particle forming solvents on signal intensity. Conditions: Dionex SFC system; 100% CO_2; column 100 × 1 mm I.D. Deltabond CN maintained at 60°C; pressure program, 3 min at 180 atm, 10 atm/min to 350 atm; injection of 0.5 μl of 400 ng/μl of caffeine or pyrene in methanol. PB conditions: nebulizing gas pressure, 45 p.s.i.; nebulizing capillary position, −2; desolvation chamber temperature, 40°C. Particle solvent flow-rate, 0.05 ml/min. Other conditions given in Experimental section.

Table 1
Properties of common solvents

	Molecular mass (a.m.u.)	Boiling point (°C)	Surface tension (dynes/cm) at 20°C
Water	18.01	100	73.1
Acetonitrile	41.05	82	29.3
Toluene	92.14	111	28.5
Cyclohexane	86.14	81	25.5
Methanol	32.04	65	22.6
Isopropanol	60.1	82	21.7
Hexane	86.2	69	18.4
Pentane	72.15	36	13.7
Carbon dioxide	44.01	−78	1.16

surface tension greater than that of methanol exhibited better sensitivity than hexane for pyrene. Thus, in this case a factor other than surface tension must be responsible. Toluene may exhibit a carrier effect for pyrene. The carrier effect is the enhancement of transmission efficiency through the PB interface due to the presence of a carrier compound which may be a mobile phase additive or a coeluting compound. The carrier effect has been reported for structurally similar carriers. [27–29] In the case of pyrene and toluene, sufficient structural similarity apparently exists for this effect to have a great impact. The carrier effect will be fully addressed in the next section.

Our data on the nature of the PFS demonstrated several criteria for the selection of a suitable solvent. Although toluene gave the best sensitivity for pyrene (not caffeine), severe problems were encountered during the use of this solvent. These problems were due to toluene's ability to leach-out O-ring greases and redeposit them on the skimmers resulting in a loss of sensitivity and an increase in background. Although hexane gave the best sensitivity irrespective of the analyte, hexane may not be used under most conditions since it contributes to the background at molecular mass 86. Numerous compounds of interest have significant ions below this range, therefore, hexane is not an appropriate choice. Another constraint on PFS is solvent volatility. Pentane is so volatile (surface tension lower than hexane) that it contributes very little to particle formation. Thus a poor response with this solvent was obtained.

Composition of the particle forming solvent

Winkler estimated that his version of the PB interface was about 5% efficient in analyte transport [24]. Since then Van Der Greef et al. have determined that the transport efficiency is in the range of 16%. [30] The major analyte losses occur in the desolvation chamber in the region prior to the nozzle, in the momentum separator on the skimmers, and in the transfer tube [24]. In order to minimize the effects of these regions in PB, careful interface redesign considering flow dynamics and the 'stickiness' of the surfaces may be necessary. However, one can alter the transport efficiency of the momentum separator without redesign. Since for the momentum separator the transport of analytes improves with increased momentum, increasing the weight of particles should result in an improved transport efficiency and thus sensitivity. To this end the carrier effect has been utilized.

The carrier effect has been described as the formation of a 'complex' of a chemical or physical nature between the analyte and an additive in the mobile phase or a coeluting compound that results in improved sensitivity and linearity [31,32]. To date the carrier effect has been demonstrated with carriers exhibiting the following characteristics: capability of hydrogen bonding, structurally similar to the analyte, or an isotopically labeled analogue of the analyte [27–

29,31–34]. The carrier effect is an attractive option because these 'complexes' are easily broken in the environment of the hot ion source. Thus, for analytes capable of 'complex' formation, an increase in sensitivity, improved linearity, and artifact-free spectra have been observed. However, there are limitations on carriers such as volatility, solubility, and spectral interference. Volatility is required for robust operation of the PB interface because the nozzle/skimmer should not plug due to carrier accumulation. In order to participate in particle formation the carrier has to be soluble in the PFS. And lastly the carrier should afford spectral contribution in regions of little consequence to the analytes of interest. One such carrier that meets these criteria is ammonium acetate (NH_4OAc). Fig. 5 demonstrates the effect of NH_4OAc concentration for two analytes: caffeine and pyrene. For caffeine, the data clearly show a carrier enhancement factor of 1.87 with 0.01 *M* NH_4OAc in methanol relative to pure methanol. Whereas no improvement was observed for pyrene since no 'complex' by hydrogen bonding between buffer and analyte is feasible. Within experimental error, similar results for caffeine were obtained by Budde et al. under LC–PB-MS conditions [32]. The utility of carrier effect in SFC–PB-MS will be demonstrated in the Applications section for the analysis of carbamate pesticides.

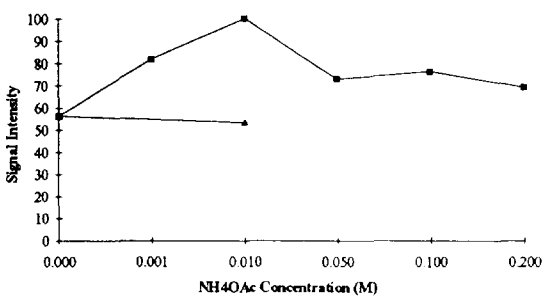

Fig. 5. Effect of NH_4OAc concentration in particle forming solvent on signal intensity. Conditions: Dionex SFC system; 100% CO_2; column 100 × 1 mm I.D. Deltabond CN maintained at 60°C; pressure program, 3 min at 150 atm, 10 atm/min to 350 atm; injection of 0.5 μl of 400 ng/μl of caffeine (■) or pyrene (▲) in methanol. PB conditions: nebulizing gas pressure, 45 p.s.i.; nebulizing capillary position, −2; desolvation chamber temperature, 40°C. Particle forming solvent flow-rate, 0.05 ml/min. Other conditions given in Experimental section.

3.3. Chromatographic parameters

Mobile phase flow-rate

The PB interface dependence on the mobile phase flow-rate is an important consideration in terms of applicable column sizes (I.D.). As shown in Fig. 6, mobile phase flow-rate was found to have no effect up to 1.1 ml/min. This working range up to 1.1 ml/min allows for coupling of packed columns of 2.1 mm I.D. and smaller. Beyond 1.1 ml/min the sensitivity decreased to about 20% at 2.0 ml/min. The decrease in sensitivity was attributed to poor nebulization efficiency because of the visible dispersion of the aerosol. This dispersion of aerosol was greatest at 2.0 ml/min at which point some droplets were impacting on the viewing window which is located above the nebulizer. No significant changes in the ion source pressure [$2–3 \cdot 10^{-5}$ Torr (1 Torr = 133.322 Pa)] was observed while the mobile phase flow-rate was changed up to 2.0 ml/min CO_2.

Mobile phase composition

As discussed in our previous paper [8], one of the disadvantages of operating without the PFS was the dependence of sensitivity on mobile phase composition. It was found that optimum sensitivity occurred at 4% methanol-modified

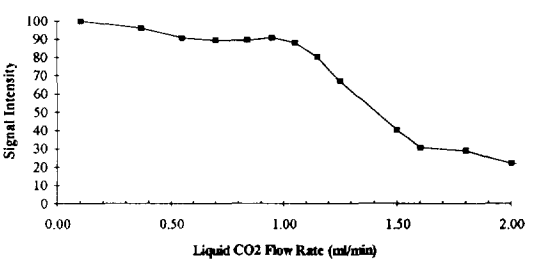

Fig. 6. Effect of liquid CO_2 flow-rate on signal intensity. Conditions: ISCO SFC system; 100% CO_2. FIA of 0.5 μl of 200 ng/μl of caffeine in methanol. PB conditions: nebulizing gas pressure, 45 p.s.i.; nebulizing capillary position, −3; desolvation chamber temperature, 40°C. Particle forming solvent flow-rate, 0.02 ml/min methanol. Other conditions given in Experimental section.

CO_2 [8]. This fact creates a non-ideal situation where method development (which may involve modifier concentration changes) and sensitivity are in direct conflict. Thus, one of the rationales for using PFS was to minimize or eliminate the link between sensitivity and mobile phase composition. For the current system employing the PFS, the hypothesis was that no more methanol would be necessary for efficient nebulization and droplet formation. Mobile phase composition was assessed from 0 to 8% methanol in CO_2 in increments of 2%. The results (data not shown) indicated no effect of modifier concentration on sensitivity within the error of the technique. Consequently, method development in SFC, utilizing step gradient or continual gradient composition ramps, would not suffer the penalty of loss in sensitivity unlike the case of our previous system [8].

3.4. Quantification

After optimization of the above discussed parameters, the sensitivity performance of the SFC–PB-MS system was evaluated. Caffeine was chromatographed isobarically and isoconvertically with 4% methanol-modified CO_2. The PFS was 0.01 *M* NH_4OAc in methanol. Data for both single ion monitoring (SIM) and scan were acquired with an ion source temperature at 250°C. The mass spectrometer was calibrated with perfluorotributylamine (PFTBA) and tuned to maximize the 219 a.m.u. peak of PFTBA. The obtained calibration curve for full scan data (150 a.m.u. wide) yielded a correlation coefficient of 0.9978 and the LOD was experimentally found to be 10 ng injected at a signal to noise ratio of 3. Fig. 7, shows the signal from a triplicate injection of caffeine at 10 ng. The scan data detection limit reported here is 10 to 100 times lower than the best reported estimates by Edlund and Henion [18] and Browner et al. [19,20] and a factor of 4 to 5 lower than our previous

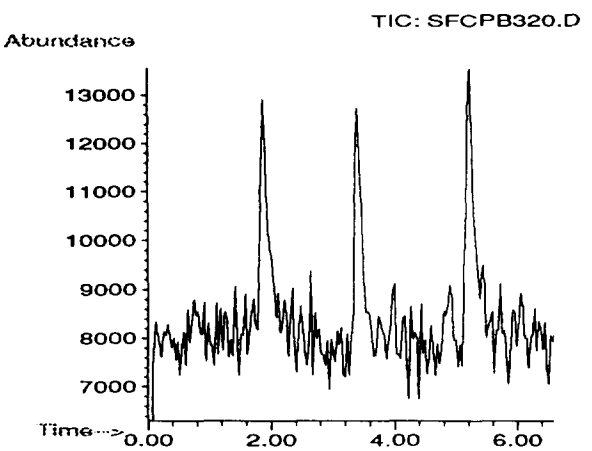

Fig. 7. Chromatogram of 3 replicate injections of caffeine at 10 ng. Conditions: Dionex SFC system; 4% methanol-modified CO_2; column 100 × 1 mm I.D. Deltabond CN maintained at 60°C; pressure 350 atm; PB conditions: nebulizing gas pressure, 45 p.s.i.; nebulizing capillary position, −2; desolvation chamber temperature, 40°C. Particle solvent flow-rate, 0.02 ml/min 0.01 *M* NH_4OAc in methanol. Other conditions given in Experimental section.

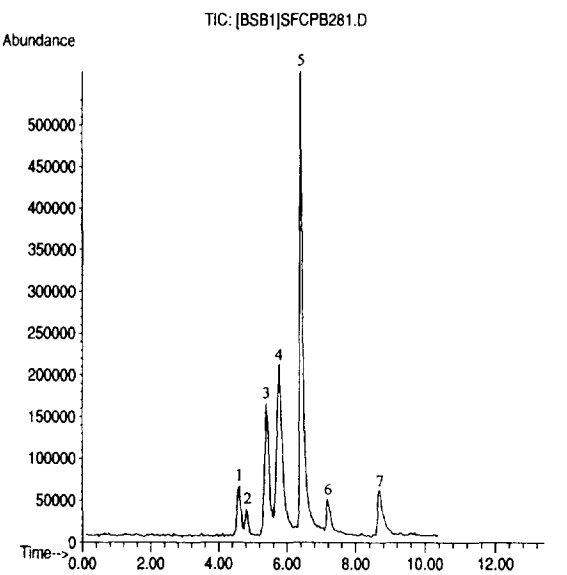

Fig. 8. Separation of carbamate pesticides using EI-MS. Peaks are as follows: 1 = baygon, 2 = aldicarb, 3 = carbofuran, 4 = methiocarb, 5 = carbaryl, 6 = methomyl, 7 = oxamyl. Conditions: Dionex SFC system; column 250 × 1 mm I.D. Hypersil Si maintained at 50°C; 4% methanol-modified CO_2; pressure program: 6 min at 145 atm, 145 to 400 atm in 2.0 min; flow-rate, 0.20 ml/min liquid flow; injection 0.5 μl of 2.0 μg/μl per component; PB conditions: restrictor (linear, 25 μm I.D.) position, −4; desolvation chamber temperature, 40°C; nebulizing gas pressure, 40 p.s.i.; particle forming solvent, 0.02 ml/min methanol. Other conditions given in Experimental section.

results. The calibration curve for extracted ion chromatogram (EIC) for the 194 molecular ion of caffeine was characterized by a correlation coefficient of 0.9984 and the LOD was 5 ng. The data for SIM had a correlation coefficient of 0.9986 and a LOD of 1 ng caffeine injected at a signal to noise ratio of 3. The reproducibilities (RSD) for the integrated signal ranged from 2 to 9% RSD at the high and low ends of the calibration plots.

3.5. Applications

The analysis of carbamates demonstrates the utility of SFC–PB-MS to identify compounds of significant environmental interest. Separation of seven thermally labile carbamate pesticides within 10 min with near baseline resolution is shown in Fig. 8. Fig. 9 shows the background subtracted and library spectra of baygon ($M_r = 209$). The spectra of all carbamates (data not presented)

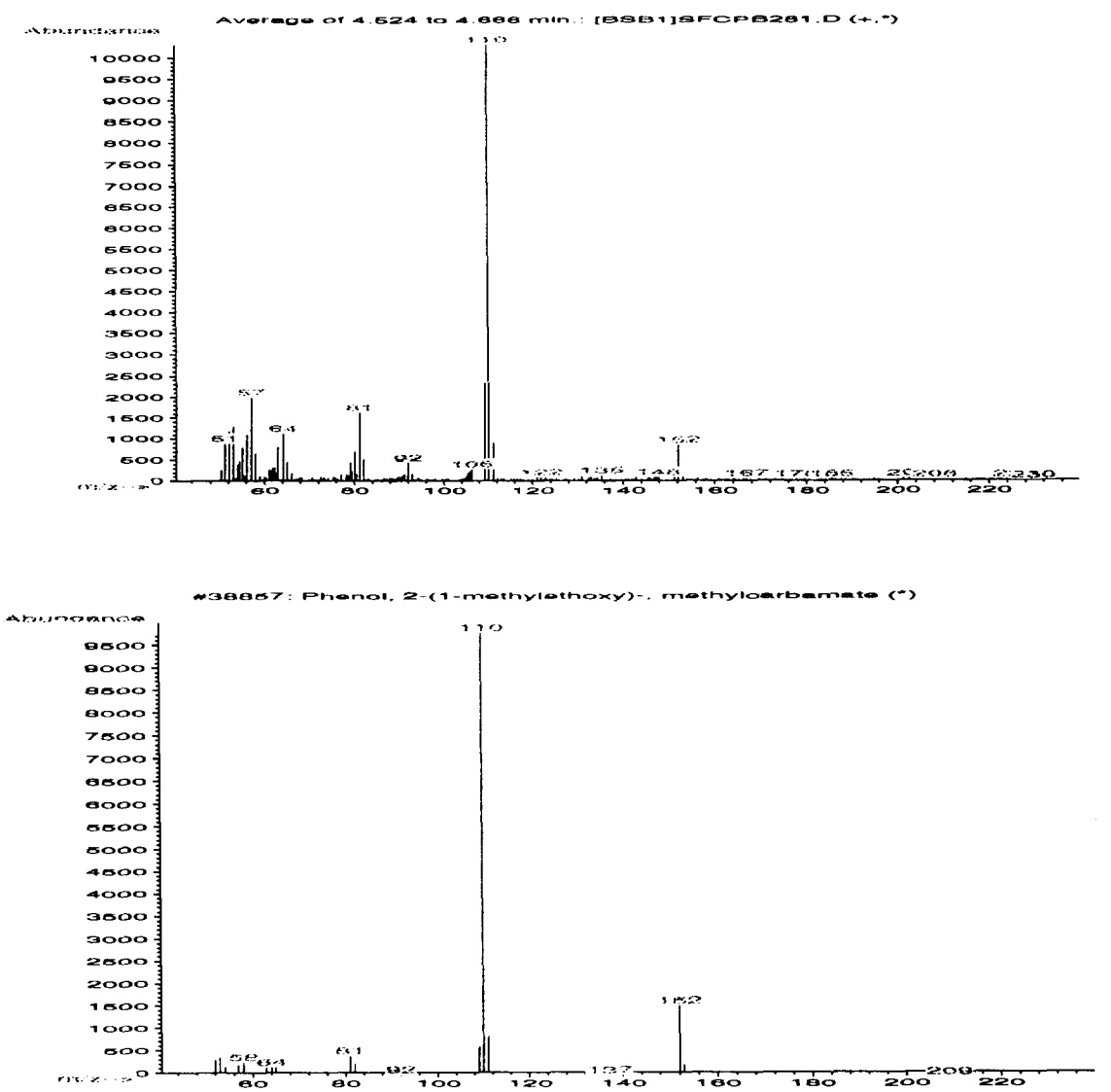

Fig. 9. EI spectrum of baygon (top) and library spectrum (bottom).

Table 2
Particle beam enhancement factors from the addition of 0.01 *M* NH_4OAc in methanol as particle forming solvent

Pesticide	Enhancement factor
Baygon	1.01
Aldicarb	1.01
Carbofuran	1.01
Methiocarb	1.33
Carbaryl	1.51
Methomyl	1.55
Oxamyl	1.54

show the characteristic lack (or less than 10% intensity) of the molecular ion peak and extensive fragmentation. Since some of these carbamates are capable of hydrogen bonding, a study was performed to determine the carrier enhancement factor for these compounds. The carrier enhancement factors with 0.010 *M* NH_4OAc in methanol relative to pure methanol are shown in Table 2. As indicated by the data, the first three pesticides showed no improvement, whereas the last four showed an increase in response from 1.33 to 1.55 over pure methanol as the PFS. The lack of a molecular ion hampers compound identification for these compounds. This problem may be solved by employing a 'softer' ionization technique such as chemical ionization (CI). Use of methane CI is characterized by the presence of a significant molecular ion peak at $[M+H]^+$. The same carbamate pesticides as shown in Fig. 8 were separated and detected under CI (data not shown). Fig. 10 shows the CI spectra of the baygon.

One of the limitations of our previous system which did not use PFS was the lack of detection of analytes eluted with pure CO_2 mobile phase [8]. This limitation hampers method development in SFC. The lack of detection of analytes under pure CO_2 conditions is primarily due to inadequate particle formation. In the current system, particle formation is aided by the presence of the PFS. Thus acquisition of chromatograms utilizing pure CO_2 should be readily feasible. The separation of five PAHs (Fig. 11) demonstrates this added capability. EI spectra (data not shown) characteristic of PAHs are typified by the presence of the molecular ion peak as the base peak and the presence of $[M/2]^+$ ions.

4. Conclusion

The SFC–PB-MS system aided with particle forming solvent was evaluated for packed col-

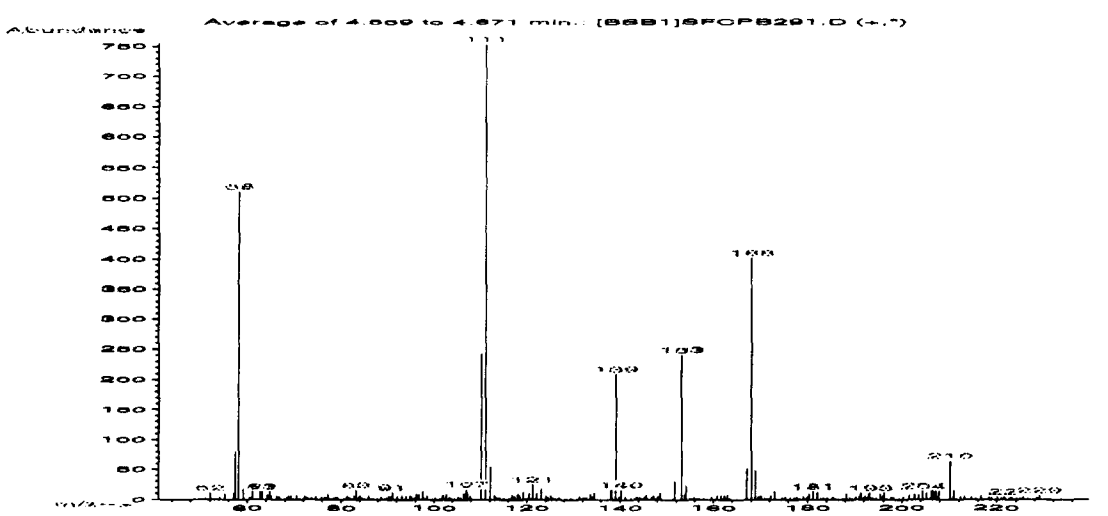

Fig. 10. CI spectrum of baygon.

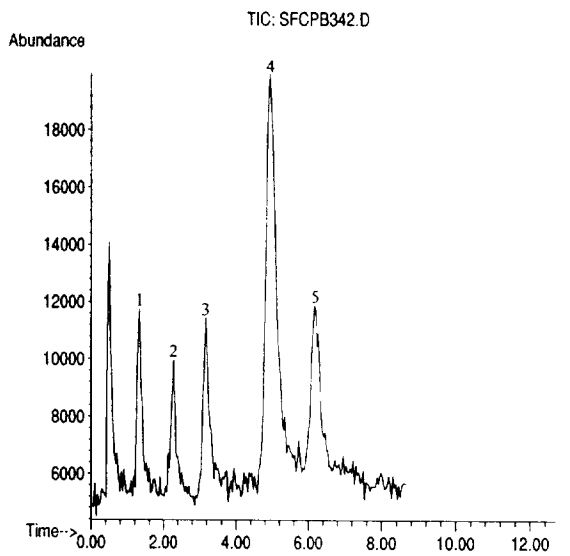

Fig. 11. Separation of polyaromatic hydrocarbons. Peaks are as follows: 1 = anthracene, 2 = pyrene, 3 = chrysene, 4 = benzo[*k*]fluoranthene, 5 = benzo[*a*]pyrene. Conditions: Dionex SFC system; column 100 × 1 mm I.D. ODS maintained at 60°C; pure CO_2; pressure program: 1 min at 250 atm, 5.0 atm/min to 300 atm, 0.20 ml/min liquid flow; injection 0.5 μl of 200 ng/μl per component; PB conditions: Restrictor (linear, 25 μm I.D.) position, −4; desolvation chamber temperature, 40°C; nebulizing gas pressure, 40 p.s.i.; particle forming solvent, 0.04 ml/min hexane. Other conditions given in Experimental section.

umn SFC–MS coupling. The effects of various operational parameters were characterized.

It was experimentally determined that the optimal aerosol formation and consequently the transport efficiency were maximized with a 50 μm I.D. nebulizing capillary with a PFS flow-rate of about 0.035 ml/min. Unlike the system described in our previous paper [8], the performance of this system was found to be unaffected by the concentration of methanol-modified CO_2 in the mobile phase. A separation of PAHs with 100% CO_2 has demonstrated this flexibility in mobile phase composition. The effect of mobile phase flow-rate was assessed and found to have no effect (up to 1.1 ml/min) thus permitting the use of up to 2.1 mm I.D. packed columns. The transport efficiency of the PB interface was improved by the nature and composition of the PFS as exhibited by the enhanced signal intensities (for both caffeine and pyrene) with hexane. The carrier effect reported under LC–MS conditions was also explored. With the present system, the carrier effect contributed to the linearity of the calibration curves as well as detection of analytes (caffeine, pyrene, and carbamate pesticides). The quantitative performance of the current SFC–PB-MS was found to be improved over previous reports. The EI LODs of the system for SIM and scan were determined to be 1 and 10 ng for caffeine, respectively. These LODs are 4 to 5 times better than the values obtained without the PFS in our previous paper, and these scan LODs are 10 to 100 imes better than the previous estimates of Edlund and Henion [18] and Browner et al. [19,20] The separation of thermally labile carbamates demonstrated the mild conditions operating under SFC and speed and selectivity of the method.

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