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A Simple, Convenient, and Efficient Preparative GC System that Uses a Short Megabore Capillary Column as a Trap

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Abstract A simple, convenient, and highly efficient preparative GC system has been developed that uses short sections of megabore capillary columns as sample collection (sorbent) traps. The performance of this system with various types of capillary column traps and under various collection conditions was systematically investigated with model compounds, including C4 to C20 normal alkanes, esters, and alcohols. The thickness and polarity of the sorptive stationary phase and the temperature of the collection trap affected trap performance. Each group of compounds was efficiently trapped above a critical Kovat's index, and the type of trap (deactivated, methyl polysiloxane, polyethylene glycol), film thickness, and whether or not the trap was cooled significantly shifted this threshold index. Above this critical index, recovery efficiencies of traps with methyl polysiloxane films were 80–100% for a wide range of injected sample mass. For example, a DB-1 collection trap with a film thickness of 1.5 μm methyl polysiloxane operated at ambient temperature trapped >84% of the mass of injected compounds of all three chemical classes with Kovat's index >1,100 (determined on a nonpolar column) with injected sample mass ranging from 10 to 1,000 ng of each compound. This preparative GC system is technically and economically feasible for most researchers. Furthermore, it is suitable for the preparation of NMR samples of volatile and semivolatile compounds, especially with sample sizes ranging from several nanograms to several micrograms.

Keywords Preparative GC · Megabore capillary column · Open tubular trap · Semiochemicals · Fractionation · Isolation · Purification · NMR · Sample preparation

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Introduction

The identification of semiochemicals is an essential step for understanding chemically mediated behavioral and ecological interactions. Numerous substances have been identified, and the databases of these compounds have grown exponentially in recent years. Nonetheless, identification of semiochemicals, some of which occur in trace amounts, remains an arduous but indispensable prelude for the ensuing basic and applied research.

The isolation and purification of active compounds are key steps in a bioassay-guided identification process, and preparative GC is a powerful purification technique for volatile and semivolatile compounds (Heath and Dueben 1998). However, there are several technical and practical constraints associated with preparative GC: (1) the necessity to condense volatile compounds by cooling the collection trap with a refrigerant, commonly dry ice, dry ice/acetone, or liquid nitrogen (Heath and Dueben 1998), a rather inconvenient process that makes rapid exchanges of traps cumbersome and time-consuming; (2) low, unsatisfactory recovery efficiency of volatile compounds; and (3) expense and difficulty of setting up a commercial instrument or of fabrication of a custom-built system.

Capillary collection traps, or open tubular traps (OTT), have been used to enrich and concentrate volatile organic compounds from the headspace of various samples for GC/

GC-MS analyses of environmental and food samples (Baltussen et al. 2002; Pillonel et al. 2002; Kloskowski et al. 2007). This technique was first developed by Grob and Habich (1985), and later, Blomberg and Roeraade (1987) applied it to volatile collection from preparative GC with the advantage of high recovery efficiencies of volatiles without cryogenic trapping. They suggested that this preparative GC technique could be an attractive approach for many applications. However, in the last two decades, there has been limited practical application of their innovation (Shimoda et al. 1993, 1996).

The parameters involved in sample collection for both instrumental analyses and preparative GC by using OTT appear to be the same. Although many parameters that influence the trapping efficiency of OTT for sample enrichment have been studied (Grob and Habich 1985; Burger and Munro 1986, 1987; Blomberg and Roeraade 1987; Cao and Hewitt 1992; Zhirong et al. 1999; Pettersson et al. 2004), most previous methodological studies were done with custom-made super thick film OTT along with custom-made sample collection and desorption devices and custom-modified instruments mainly for gaseous and solvent-like highly volatile compounds such as environmental pollutants. For example, Blomberg and Roeraade (1987) used custom-made 200 cm, 80 μm silicon film collection traps for preparative GC for compounds ranging from solvent-like to C12 hydrocarbon. In practical applications, however, it is convenient and cost-effective to use commercially available materials with standardized parameters that should yield more consistent results. Moreover, there has not been extensive and systematic evaluation of OTT performance for preparative GC collection especially for volatile and semivolatile semiochemicals.

Nojima et al. (2004) previously reported on a manual preparative GC system, which is relatively simple and inexpensive and achieves high recovery efficiency by using cryogenic trapping with a short section of a deactivated megabore capillary tube. This system has facilitated the efficient purification of semiochemicals for NMR analysis even at the submicrogram scale and has been used for structure elucidation of a few micrograms of a thermally and chemically unstable female sex pheromone of the German cockroach (Nojima et al. 2005). Nevertheless, this preparative system requires some familiarity with instrumentation, a refrigerant for cryogenic trapping, and a major drawback is that it is not suitable for sequential fractionation of multiple compounds within a single GC run.

In this study, we report a further improvement of this preparative GC approach that consists of a simple modification of a regular GC system coupled to an OTT sample collection by utilizing only commercially available materials and systematic evaluation of the system by using a wide range of model compounds.

Methods and Materials

Chemicals Straight chain methyl esters (C4–C16), hydrocarbons (C7–C20), and alcohols (C4–C16) were obtained from Supelco (Bellefonte, PA, USA) or Aldrich (Milwaukee, WI, USA).

Preparative GC

Modification of the GC An HP5890 gas chromatograph was converted to a preparative GC. Schematic diagrams of the preparative GC configuration and outlet port assembly are shown in Fig. 1. A split–splitless (S/SL) injection port assembly equipped with heat sink, heater cartridge, and sensor was used for the preparative GC outlet port and installed adjacent to the FID port. The heater and sensor were connected to the control ports assigned to detector B so that the temperature of the preparative GC outlet port could be controlled and monitored as "detector B."

The S/SL injection port was modified to an outlet port for the preparative GC as follows (Fig. 1a–c). The septum well on the weldment nut, which is welded to 1/16″ tubing

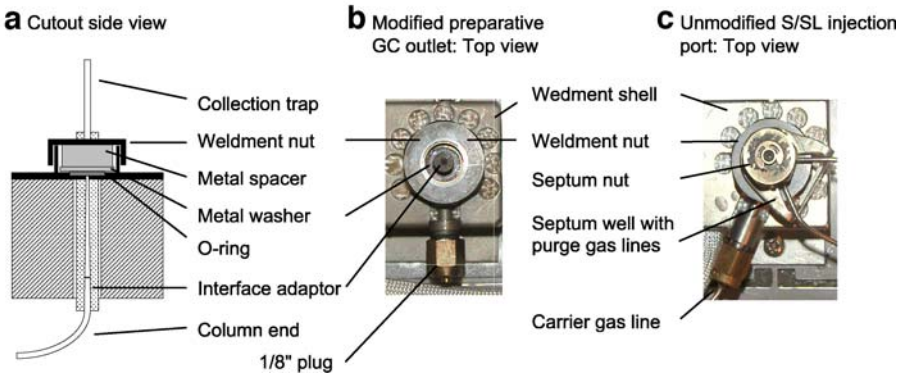

Fig. 1 **a–c** Schematic diagrams of the preparative GC configuration and outlet port assembly

for the purge gas and retained on the weldment nut by a retainer ring, was removed from the injection port. The metal spacer, used for supporting the weldment nut against the septum well, was kept and utilized for fixing an interface adaptor between the column end and collection traps. The carrier gas line welded to the shell weldment was sealed with a 1/8″ stainless steal plug (Swagelok®) (Fig. 1b).

Installation of a megabore capillary column The distal column end was cut with a column cutter, and finger lipids and other contaminants were removed by wiping with methanol-wetted tissue paper. The column end was inserted into the preparative GC outlet port so that its end protruded several centimeters above the port. A direct injection glass liner (1 mm Uniliner® for 0.32/0.53 mm ID, columns, 1.0 mm ID, 6.3 mm OD×78.5 mm; Restek, Bellefonte, PA, USA) was used as an interface adaptor between the column end and collection trap (Fig. 1a and b). The column was pushed into the longer tapered seat of the liner to make a securely tight seal. Then, the glass liner–column assembly was retracted into the outlet port. An O-ring for inlet glass liners for Agilent GCs (6.3–6.5 mm ID), a flat stainless steel washer (6.7 mm ID, 17.2 mm OD), and the metal spacer that was taken from the septum well were put on the liner in this order (Fig. 1a). Then, the weldment nut was put on the glass liner, finger-tightened, and then tightened with a wrench while holding the glass liner with clean forceps. It was important to avoid rotating the glass liner while tightening the weldment nut to prevent twisting and stressing the connection between the glass liner and the column. Any tension at this connection may result in an unexpected detachment of the column under frequent temperature/pressure fluctuations and vibrations from the oven fan. The end of the column was not fixed to the modified S/SL outlet port with a column nut and ferrule to provide some free rotation for the column.

Collection traps Sections (40 cm) of various types of megabore (0.53 mm ID) capillary columns were used as collection traps: (a) collection traps without stationary phase whose inner surface was deactivated (Agilent Technologies, Santa Clara, CA, USA); (b) nonpolar collection traps on which a methyl polysiloxane film was bonded at a film thickness of 0.5, 1.5, or 5.0 μm (DB-1, Agilent Technologies); and (c) a polar collection trap on which polyethylene glycol film was bonded at a film thicknesses of 1.5 μm (Stabilwax®, Restek, Bellefonte, PA, USA). The collection traps were rinsed twice with 100 μl of methylene chloride and dried at room temperature overnight. Both ends of the collection traps were cut and cleaned with methanol on tissue paper. For reuse, the traps were reconditioned by rinsing with solvent, and the trap was carefully examined for a square and even end for a secure connection to the interface adaptor.

Preparative GC conditions A nonpolar EC-5 megabore capillary column (1.0 μm film thickness, 0.53 mm ID× 30 m, Alltech Associates, Deerfield, IL, USA) was used as a separation column, and a 2-m deactivated column (0.53 mm ID, no stationary phase; Alltech Associates) was connected to the front of the separation column with a press fit connector (Alltech Associates). Helium was used as the carrier gas at a head pressure of 27.6 kPa and a flow rate of 5.5 ml min^{-1}. The oven temperature was set at 45°C for 2 min, increased at 15°C min^{-1} to 250°C, and held for 5 min. The injector and collection port temperatures were held at 270°C and 250°C, respectively. The septum purge flow rate was set at 3 ml min^{-1} with a total flow rate of 22 ml min^{-1} in the split injection mode (split ratio of 1:5), whereas the total flow rate was set at 50 ml min^{-1} in the splitless injection mode with the purge valve off for 1 min. Retention times of model compounds were established before preparative GC work by FID under the same analytical conditions. The Kovat's retention indices (Heath and Dueben 1998) of model compounds were estimated by retention times of straight chain hydrocarbons.

Sample Collection and Compound Recovery from Traps

Sample collection For collection with cryogenic trapping, a detachable and well-insulated sheath equipped with a reservoir for a refrigerant was used to cool the collection traps (Fig. 2a; see detail in Nojima et al. 2004). The reservoir cup was filled with dry ice, and a collection trap was inserted into the sheath for equilibration. To collect compounds, the trap–sheath assembly was connected to the interface adaptor in the outlet port by gently pushing the trap end into the tapered seat of the adaptor just before a sample collection window. A gentle connection gave a secure seal that resulted in high recovery efficiencies and facilitated easy, smooth, and quick exchanges of collection traps for multiple collections. A tighter connection may cause breakage of the tip of the collection trap during trap exchanges, resulting in poor recovery when column fragments were in the glass liner. We found that a syringe cleaning wire (0.17 mm OD; Hamilton, Reno, NV, USA) worked well to remove column fragments from the adaptor. During the collection, the lower end of the cooling sheath was kept in contact with the interface adaptor so that a gradual cooling zone was generated along the collection trap for better recovery efficiencies (Brownlee and Silverstein 1968). At the end of a sample collection window, the collection trap was withdrawn together with the sheath; the trap was pulled out from the sheath and set up on a lab

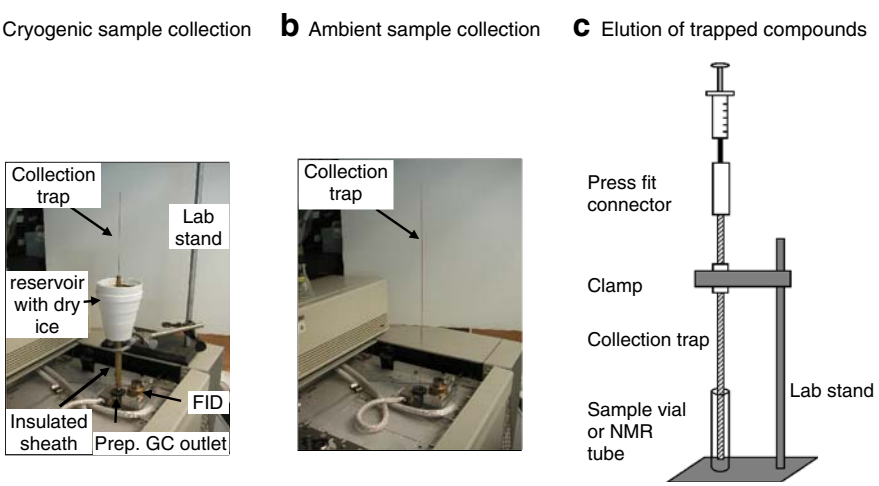

Fig. 2 Sample collections under cryogenic (**a**) and ambient (**b**) conditions, and sample elution from the trap (**c**)

stand for the extraction step. For a long collection, a lab stand and a clamp were used to hold the sheath (Fig. 2a).

For sample collection at ambient temperature, a clean collection trap was connected to the interface adaptor as described above. The connection between the trap and the adaptor was secure enough to hold the 40-cm collection trap without any other support (Fig. 2b).

Elution of compounds from the traps The collection trap was set up vertically on a lab stand as shown in Fig. 2c. A press fit connector was attached to the upper end of the trap, and the other end was put into a sample vial. Then, a GC syringe was used to introduce solvent containing an internal standard into the press fit connector. In a preliminary experiment, compounds trapped on various types of collection traps were fully extracted with 40 μl of hexane, methylene chloride, or ether; 10 μl methylene chloride was found to be sufficient to extract trapped compounds for qualitative analyses.

Analytical GC conditions Sample analysis was conducted on an HP5890 GC equipped with a nonpolar EC-5 capillary column (0.25 μm film thickness, 0.25 mm ID×30 m; Alltech Associates). Helium was used as the carrier gas at a head pressure of 115 kPa (flow rate, 1.5 ml min^{-1}). Oven temperature was set at 50°C for 2 min, increased at 15°C min^{-1} to 250°C, and held for 5 min. The injector and detector temperatures were set at 270°C. Septum purge flow rate was set at 3 ml min^{-1} with a total flow rate of 14 ml min^{-1} in the split injection mode (split ratio of 1:10), whereas a total flow rate was set at 50 ml min^{-1} in the splitless injection mode with the purge valve off for 1 min.

Experiment 1: qualitative comparison of various types of collection traps under ambient and cryogenic conditions The trapping capabilities of deactivated capillary tubes (0 μm film), DB-1 capillary tubes with a film thickness of 1.5 μm, and Stabilwax® capillaries with a film thickness of 1.5 μm were compared. A mixture of model compounds that included straight chain methyl esters (C4–C16), hydrocarbons (C7–C20), and alcohols (C4–C16) in methylene chloride at a concentration of 300 ng μl^{-1} each was used for this experiment. One microliter of the mixture was injected into the preparative GC in split mode (split ratio of 1:5) so that the C4 ester, methyl butanoate, separated from the solvent peak that obscured it in the splitless injection mode. The collection window was 2 to 18.5 min, so that all compounds from a single injection were collected in the same collection trap (Fig. 3). The collections were made both under cryogenic and ambient conditions. Trapped compounds were eluted with 10 μl methylene chloride, and 2 μl of each extract were analyzed in split mode at a split ratio 1:5. Two replicates were made for each trapping condition.

Experiment 2: qualitative comparison of various film thicknesses of DB-1 collection traps under ambient and cryogenic conditions. The same collections were made using DB-1 capillary traps with film thicknesses of 0.5, 1.5, and 5.0 μm. Trapped compounds were recovered and analyzed as described above.

Experiment 3: quantitative comparison of the trapping efficiencies of various film thicknesses of DB-1 collection traps at ambient temperature. Mixtures of model compounds at concentrations of 10, 100, and 1,000 ng μl^{-1} of each compound in hexane were used for this experiment. For deactivated collection traps (0 μm film), a mixture of C13–C16 methyl esters, C16–C19 hydrocarbons, and C13–C16 alcohols was used, whereas for DB-1 collection traps (which exhibited better trapping capabilities in preliminary studies), a mixture of C8–C11 methyl esters, C11–C14 hydrocarbons, and C8–C11 alcohols was used. One microliter of each mixture was injected into the preparative GC in

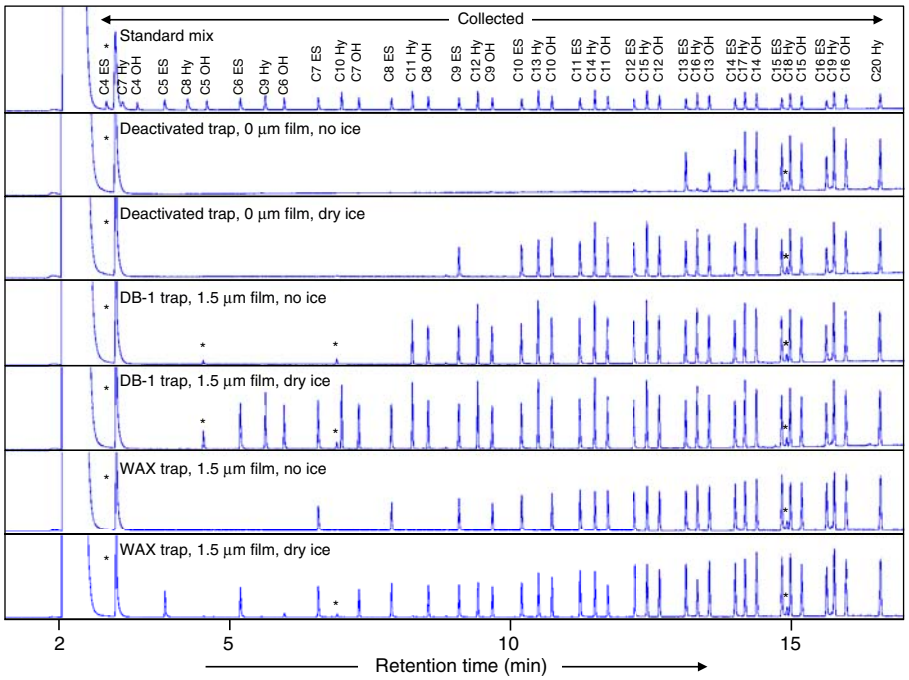

Fig. 3 A comparison of trapping of model compounds by three types of collection traps under ambient and cryogenic conditions. One microliter of a mixture of straight chain methyl esters (C4–C16), hydrocarbons (C7–C20), and alcohols (C4–C16) at a concentration of 300 ng μl^{-1} per compound was injected into the preparative GC in split mode, and all compounds were collected in a single collection trap between 2 and 18.5 min of each run. Collections were made with both dry ice and at ambient temperature. Trapped compounds were eluted with methylene chloride, and the extracts were analyzed by GC-FID. *Asterisks* indicate impurities that likely originated from the instrument, the solvent, and not from the traps (see text)

splitless mode, and all compounds in the mixture were collected in the same collection trap during each run. The collection windows were 13–18 min for deactivated collection traps and 8–13.5 min for DB-1 collection traps. All collections were made at ambient temperature. Trapped compounds were eluted with 20 µl hexane, then 10 µl hexane containing 500 ng pentacosane internal standard, and then an additional 20 µl hexane. Extracts were combined, and 1 µl of each sample was subjected to quantitative (FID) GC analysis in splitless mode. Five replicates were made for each trap condition.

Experiment 4: qualitative analysis of distribution of trapped compounds in the collection traps. We used the same mixtures of compounds at a concentration of 1,000 ng μl^{-1} each compound, and the same conditions described above for deactivated and DB-1 collection traps. After the collections, each 40 cm collection trap was cut into 10 cm sections, and each was eluted with 20 µl hexane, then 10 µl hexane containing 500 ng pentacosane internal standard, and then an additional 20 µl hexane. One microliter of each extract was analyzed by GC-FID in splitless mode. Two replicates were made for each collection trap.

Experiment 5: a practical collection trial. A mixture of C4–C12 methyl esters, C7–C15 hydrocarbons, and C4–C12 alcohols in methylene chloride, at a concentration of 300 ng μl^{-1} each compound, was used for this experiment. One microliter of this mixture was injected into the preparative GC in splitless mode, and compounds were collected in groups of similar retention index (a C_n methyl ester, $C_{(n+3)}$ hydrocarbon and C_n alcohol; Fig. 7), except for the last three groups that were collected together in the same DB-1 trap with 1.5 µm film thickness. Each collection window for a group was about 1 min except for the last three groups. Trapped compounds were eluted with 20 µl hexane, and 1 µl of each extract was injected into a GC-FID in split mode at a split ratio 1:16.

Results and Discussion

Experiment 1: Qualitative Comparison of Various Types of Collection Traps under Ambient and Cryogenic Conditions

Trapping of model compounds in megabore collection traps varied with trap type and whether or not we used cryogenic conditions (Fig. 3). Cooling the collection traps with dry ice greatly increased the trapping capabilities of various types of collection traps. At ambient temperature, a deactivated megabore column (0 µm film) failed to trap all three compound types at or below a retention index of about 1600 (on an EC-5 column), but with cryogenic trapping the same trap was effective down to 1300. Addition of a nonpolar stationary phase (DB-1, 1.5 µm film thickness) extended the range of compounds trapped down to 1100 without cooling and to 900 with dry ice. Although a polar megabore collection trap (Stabilwax®, 1.5 µm film thickness) was less effective at trapping hydrocarbons than the DB-1 trap, the WAX trap effectively trapped the C7 ester (retention index 1000) at ambient temperature and down to a C5 ester (retention index 800) with dry ice (Fig. 3). Thus,

the presence of a stationary phase greatly improved the trapping capabilities of collection traps, and cryogenic conditions (with or without stationary phase) extended the range of compounds trapped to a lower retention index. As in gas chromatography, it appears that both the affinity of compounds to the film and their volatility play important roles in the effectiveness of WAX collection traps. Although the WAX trap was more effective for esters, DB-1 traps are more suitable for practical use because this nonpolar stationary phase effectively traps highly volatile compounds and is less selective of the three chemical classes we tested. Moreover, bonded DB-1 columns tolerate both high temperatures and repeated solvent extractions.

It is important to note that even without cooling the collection trap, the addition of a stationary phase to the megabore traps vastly improved their trapping effectiveness of early eluting compounds, which practically covers a wide range of volatile–semivolatile semiochemicals. The stationary phase alleviates the need to use a refrigerant, which has been a rather inconvenient process that makes rapid exchanges of traps cumbersome and time-consuming.

Contamination is often a serious issue in preparative GC, especially when collecting minute amounts of sample. Several minor contaminants (asterisks in Fig. 3; see also experiment 2; Fig. 4) likely originated from the instrument or extraction solvents and not from traps because the contaminants became negligible after repeated sample processing (see Figs. 6 and 7).

Experiment 2: Qualitative Comparison of Various Film Thicknesses of DB-1 Collection Traps under Ambient and Cryogenic Conditions

We compared the trapping effectiveness of various film thicknesses of DB-1 collection traps. Under ambient trap conditions, a collection trap with a film thickness of 5.0 μm consistently trapped compounds of retention index 1000 and above, whereas 1.5 and 0.5 μm films trapped above 1100 and 1200, respectively (Fig. 4). All three film thicknesses trapped smaller hydrocarbons and alcohols, but not esters. The addition of cryogenic conditions extended the range of compounds trapped by 2C units to retention indices of 800 for 5.0 μm, 900 for 1.5 μm, and 1000 for 0.5 μm films. Thus, traps with thicker films were more effective than traps with thinner films. However, the addition of more stationary phase to traps already containing the same phase yielded only moderate benefits compared to the initial addition of a sorptive phase to traps without film or the choice of polar or nonpolar phase. Nevertheless, with dry ice cooling, the effectiveness of DB-1 collection traps with a film thickness of 5.0 μm extended to solvent-like compounds, C5 ester, C8 hydrocarbon, and C5 alcohol. Based on these results, we expect that thicker films on WAX collection traps will also extend the trapping effectiveness of polar compounds that elute earlier than C4 alcohol.

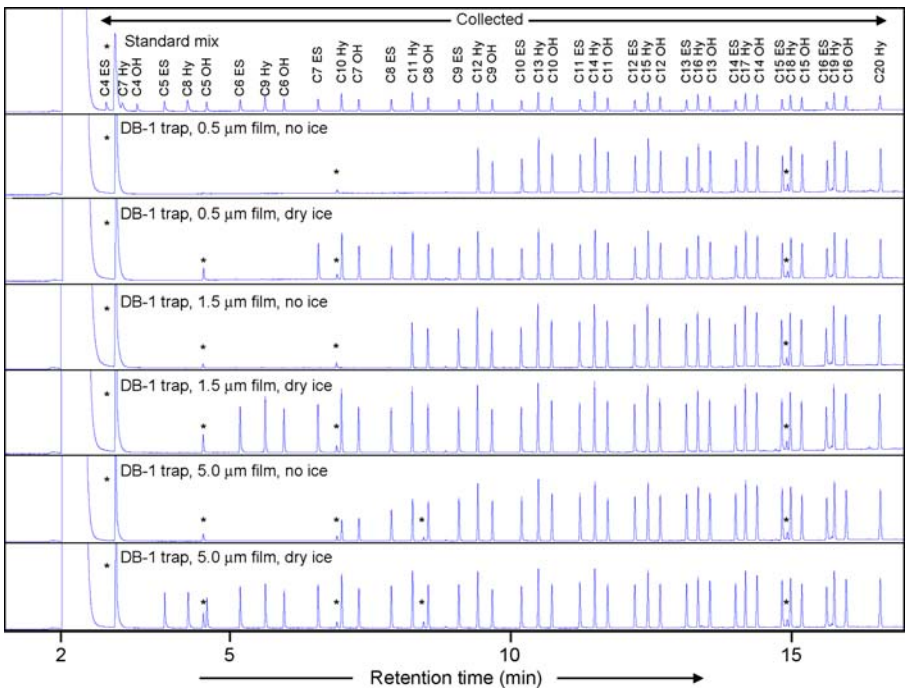

Fig. 4 A comparison of trapping of model compounds on DB-1 traps with various film thicknesses. The same mixture as in Fig. 3 was used for this experiment. The mixture was injected into the preparative GC in split mode, and all compounds were collected in a single collection trap between 2 and 18.5 min of each run. Collections were made with both dry ice and at ambient temperature. Trapped compounds were eluted with methylene chloride, and the extracts were analyzed by GC-FID. *Asterisks* indicate impurities that likely originated from the instrument, the solvent, and not from the traps

Experiment 3: Quantitative Comparison of the Trapping Efficiencies of Various Film Thicknesses of DB-1 Collection Traps at Ambient Temperature

The recovery efficacy of deactivated traps (0 μm film) varied with chemical class and sample loading (Fig. 5a). With injections of 1000 ng per compound, these traps efficiently collected >92% of all three chemical classes tested at and above the retention index of 1600. However, early eluting compounds exhibited a clear dose–response with poor trapping at low sample loadings. For alcohols, this pattern extended for all compounds in the mixture, from C13 to C16 ($P<0.05$, ANOVA). There are two main explanations for this observation. First, irreversible adsorption of alcohols to the inner surface of the deactivated trap might occur, and it would be more significant at lower sample loading sizes. Second, at high sample loading, the early eluting trapped compounds may form a stationary phase on the deactivated column, thus retarding the loss of other compounds.

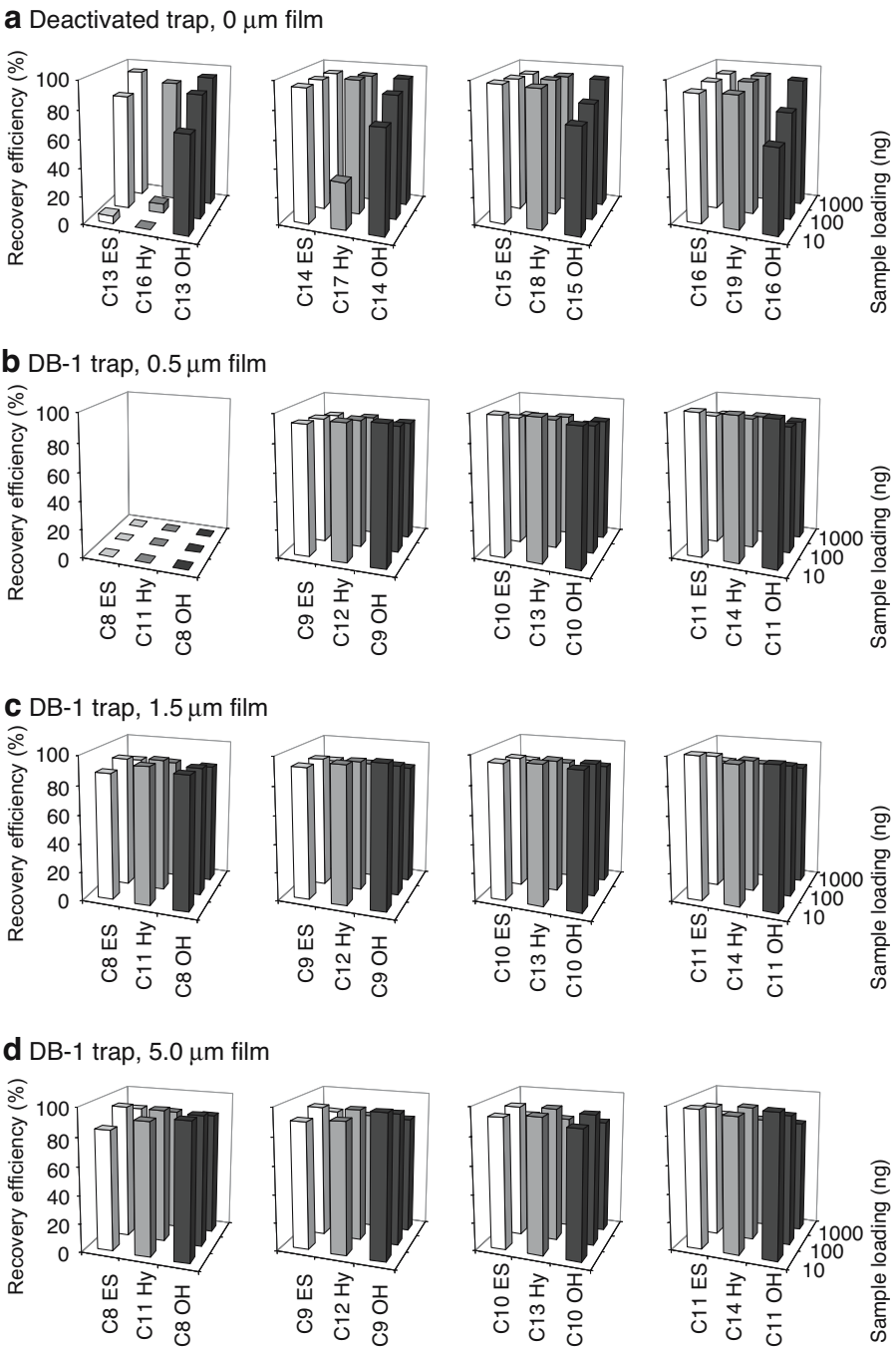

Fig. 5 a–d Trapping and recovery efficiencies of DB-1 traps with various film thicknesses and at different sample loadings conducted at room temperature. A mixture of straight chain methyl esters (C13–C16), hydrocarbons (C16–C19), and alcohols (C13–C16) was used with deactivated traps (0 μm film), and another mixture of methyl esters (C8–C11), hydrocarbons (C11–C14), and alcohols (C8–C11) was used with DB-1 traps. Preparative collections were made at sample loadings of 10, 100, and 1,000 ng of each compound. All compounds were collected in a single collection trap between 13.5 and 18.0 min for deactivated traps and between 8.0 and 13.5 min for DB-1 traps. Trapped compounds were eluted with hexane, and the extracts were analyzed by GC-FID

The recovery efficacies of DB-1 traps with a 0.5-μm film showed a distinct threshold retention index at about 1,200, below which almost none of the compounds were trapped (Fig. 5b). Adding more stationary phase (1.5 or 5.0 μm) greatly improved trapping efficiency to >80% for all tested compounds down to a retention index of 1100, and at all sample loadings (Fig. 5c,d). It is interesting to note that the DB-1 traps with a 1.5-μm film showed much better trap capability for C8 ester in this experiment in which the collection window was 8–13.5 min than in the qualitative experiment (#1) in which the collection window was 2–18.5 min (Fig. 4). It is probably because of the shorter collection window of this experiment than the earlier experiments (see the results and discussion of experiment 5). The threshold retention indices for the traps with 1.5 or 5.0 μm films could not be quantified but should be lower than 1000–1100 based on the results of qualitative experiments (Fig. 4). There was an overall inverse relationship between recovery efficiency of all DB-1 traps and the amount of sample injected ($P<0.05$, ANOVA). This is opposite to what was expected based on the classical Brownlee and Silverstein (1968) method in which recovery efficiencies decreased with decreasing amounts of injected sample. Nevertheless, the efficiency of trapping was 90–100% for almost all small samples, and even with injections of 1,000 ng per compound the trapping efficiency was >80%, generally considered satisfactory.

The connection between the column end and the collection trap is critical for achieving satisfactory recovery of eluting compounds in this preparative GC technique. An uneven cut on the column and trap ends can introduce reactive surfaces and a loose connection, both of which will diminish trapping efficiency. In our quantitative experiments, the recovery efficiencies were overall satisfactory through a wide range of sample loading sizes and different chemical classes, indicating that the interface adaptor system that used a direct injection glass liner between the column end and the traps was secure.

In preparative GC, it is thought that a gradual temperature gradient along the collection trap is necessary to achieve high recovery (Brownlee and Silverstein 1968) because a mist could be formed as the eluting compounds in the gas phase are rapidly cooled as they exit the hot GC, and the mist could then be propelled out through the trap by the relatively high linear velocity of capillary GC. In this experiment, the collection traps were kept at ambient temperature with no insulation before and during collections. This configuration probably resulted in a short gradual cooling zone along the trap in which compounds were quickly cooled from 250°C at the preparative GC outlet to about 25°C within the trap. However, the recovery efficacies were overall satisfactory. In principle, the trap collection at ambient temperature may be similar to the Grob splitless injection procedure whereby compounds are vaporized in the injection port and then condensed (solvent or thermally refocused) onto the head of the column to achieve narrow bands and sharp signals (Grob and Grob 1969). Nevertheless, as shown in experiments 1 and 2, a gradual cooling zone along the trap by a well-insulated sheath in combination with cryotrapping with dry ice significantly improved the efficiency of trapping.

Experiment 4: Qualitative Analysis of Distribution of Trapped Compounds in the Collection Traps

Under ambient collection conditions, in the deactivated collection trap (0 μm film), all injected compounds were condensed in the first 10 cm section of the trap (Fig. 6a). With the addition of a nonpolar stationary phase, not only the range of trapped compounds extended to a retention index of 1100 (again, better than in experiment 1 because a shorter collection window was used), but the condensed compounds were distributed farther up the trap, depending on the film thickness (Fig. 6b–d); more volatile compounds were trapped in sections farther from the GC outlet. Thus, threshold compounds that were trapped in the lowest 10 cm on a 5.0-μm stationary phase (e.g., retention index of approximately 1200) were trapped in the 10- to 20-cm zone on a 1.5-μm phase, and in the 10- to 30-cm zone on 0.5 μm DB-1 film thickness (Fig. 6b–d). Likewise, more volatile compounds, for example, with a retention index of 1100, were trapped in the 10- to 20-cm section on a 5.0-μm stationary phase, in the 20- to 40-cm zone on a 1.5-μm phase, and were not trapped at all on a DB-1 megabore trap with only 0.5 μm film thickness. This finding undoubtedly relates to greater interaction with the thicker stationary film, as also evidenced by later retention times on thicker films under otherwise identical GC analytical conditions. Therefore, for compounds with a retention index >1200, even a 10-cm section of megabore trap would yield satisfactory results in preparative GC. We have used 10 cm DB-1 traps with 1.5 μm film thickness to study the behavior of model compounds (data not shown). In practice, however, a longer trap is easier to handle and is more amenable to cryogenic trapping of solvent-like compounds. It is worth mentioning that when a mixture at a concentration of 1,000 ng per compound was injected, a narrow 10 mm band approximately 5 mm from the GC outlet became opaque as compounds condensed at the base of the traps.

Experiment 5: A Practical Collection Trial

Practical preparative GC generally targets single peaks for bioassay or analytical procedures that require pure compound (microchemical, spectrometric). The collection window is, therefore, much narrower than the 5–16.5 min used

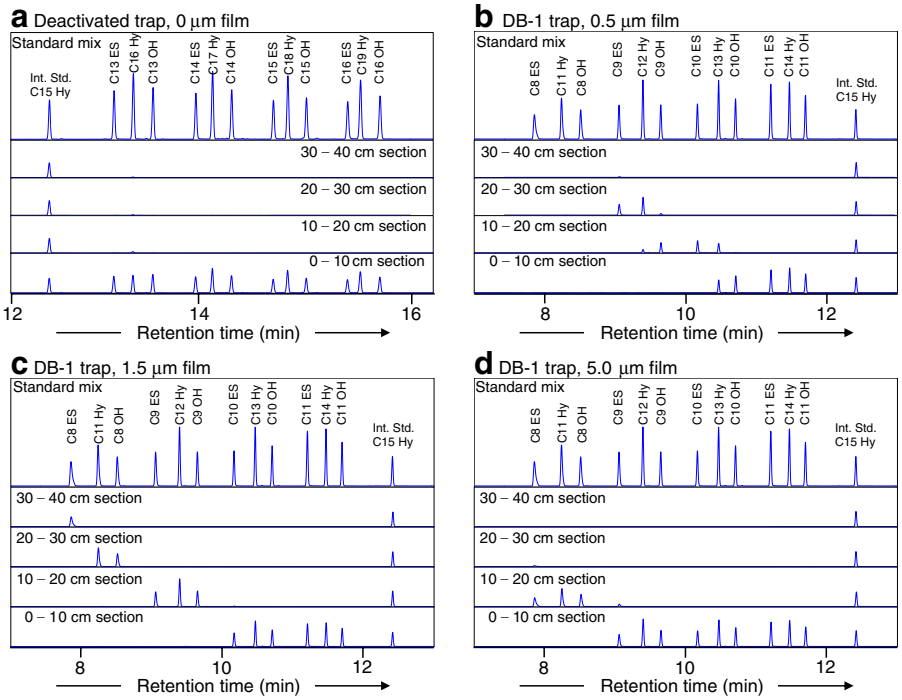

Fig. 6 a–d Qualitative analysis of the distribution of condensed compounds along the collection traps. A mixture of straight chain methyl esters (C13–C16), hydrocarbons (C16–C19), and alcohols (C13–C16) was used with deactivated traps (0 µm film), and another mixture of methyl esters (C8–C11), hydrocarbons (C11–C14), and alcohols (C8–C11) was used with DB-1 traps at a concentration of 1,000 ng μl^{-1} of each. One microliter of the mixture was injected into the preparative GC in splitless mode, and all compounds were collected in the same collection trap at each run at ambient temperature. After the collection, the 40-cm collection traps were cut into 10 cm sections and each section was extracted with hexane and subjected to GC-FID analysis

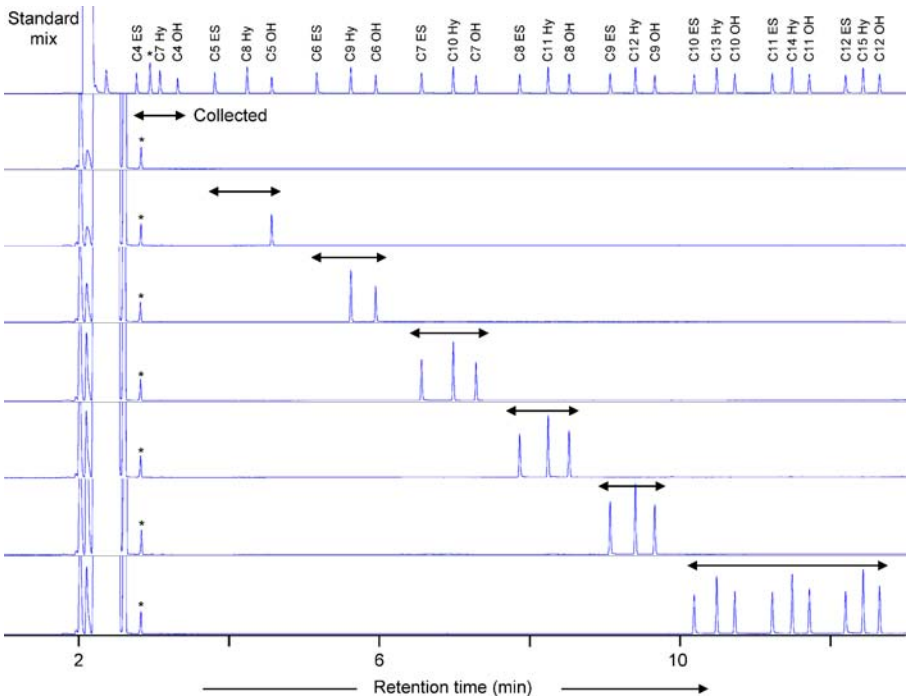

Fig. 7 A practical fractionation trial using DB-1 traps with 1.5 µm film at ambient temperature. A mixture of methyl esters (C4–C12), hydrocarbons (C7–C15), and alcohols (C4–C12) at a concentration of 300 ng μl^{-1} of each compound was used for this experiment. One microliter of the mixture was injected into the preparative GC in splitless mode, and compounds were collected in groups based on similar retention index, such that each group consisted of a methyl ester (C_n), hydrocarbon (C_{n+3}), and alcohol (C_n). The last three groups (retention index 1300 to 1500) were trapped together in the same trap. Each collection window for the groups was about 1 min except for the last three groups. Trapped compounds were eluted with hexane, and the extracts were analyzed by GC-FID. *Asterisks* indicate impurities that likely originated from the solvent and not from the traps

in the previous experiments. To verify the performance of our system with a more practical collection window, we conducted sequential fractionations of single injections by using 40 cm DB-1 megabore collection traps with 1.5 μm film. Compounds were fractionated in groups, each comprising a methyl ester (C_n), hydrocarbon (C_{n+3}), and alcohol (C_n) (Fig. 7). All collection windows for each fraction were about 1 min, except the last fraction, which was approximately 3 min. The results showed that each group was perfectly fractionated with ease and without any cross contamination (Fig. 7). The shorter collection times revealed even greater efficacy of this preparative GC approach. All three compounds around retention index 1000 were now entirely trapped, whereas this threshold was at retention index 1100 with long collection windows (see Figs. 3 and 4). Also, more volatile compounds, such as C5 alcohol, which could not be trapped even under cryogenic conditions (Figs. 3, 4), were now trapped at ambient conditions with a short collection window. It thus appears that highly volatile compounds condense in the stationary phase of the trap, but if the trap remains coupled to the GC outlet, a combination of the high velocity of the carrier gas and gradual heating of the trap might chromatograph the trapped compounds and discharge them out of the trap. In practice, on a long megabore column, the peak width near the base of early eluting compounds (i.e., collection window) is only one to several seconds, and we expect that our preparative GC system will perform even more efficiently under these conditions.

General Discussion and Conclusions

There are several technical and practical constraints associated with preparative GC, as pointed out earlier. Major advantages of our system are: (1) a wide range of volatile compounds can be effectively trapped without cooling the collection traps; (2) the collection traps can be rapidly and easily exchanged for trapping multiple but discrete GC peaks with 80–100% recovery; (3) the modification of a regular GC system into this preparative system can be achieved easily and inexpensively by using commercially available materials; and (4) commercially available capillary columns with a wide range of stationary phases, widths, and film thicknesses facilitate optimization of traps to the target compounds and to the analytical column to minimize pressure drop as compounds enter the trap. Indeed, this system could be used in a hybrid mode with longer traps with thicker film and cryotrapping for early eluting compounds and short traps with thinner film operated at ambient conditions for less volatile compounds.

A minor disadvantage of this system is that compounds are collected based on their retention times, which are determined by FID in a prior injection. Although this disadvantage is rather negligible because switching between the FID and preparative GC outlets is easy and takes only minutes, a variable or fixed splitter could be introduced at the end of the column to alleviate this problem.

In conjunction with a large-volume injection system coupled to a megabore capillary column, this preparative GC system can be used practically like a preparative HPLC system for samples up to microgram amounts. Furthermore, our preparative GC system is suitable for a simple two-step preparation of NMR samples of volatile and semivolatile compounds: a compound of interest can be optimally trapped on a short section of the megabore column and eluted directly into an NMR tube with minimal NMR solvent, resulting in high recovery of a clean sample with minimal background noise.

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