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Variable flow control and collection device for use with supercritical fluids

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Abstract

Real world samples which contain high concentrations of water and/or extractable material frequently cause intermittent or irreversible plugging of the flow control device during off-line supercritical fluid extraction (SFE). A supercritical fluid (SF) flow control/collection device has been developed which can simultaneously maintain the extraction flow-rate of the SF (±0.1 ml/min) and quantitatively (>90%) collect analytes as volatile as *n*-octane directly into an organic solvent. With this device, the extract is partially depressurized through a heated capillary restrictor and into a pressurized collection solvent, so that both temperature and pressure are used to maintain the solubility of the extract in the SF. The pressurized mixture is finally depressurized to atmospheric conditions using a backpressure regulator, so the extract can be recovered in the collection vial. Depending on the sample matrix, a restrictor heater temperature of 200°C and a backpressure regulator with a heated (>5 wt% water in sample) or unheated (<5 wt% water in sample) exit tube is required to eliminate restrictor plugging. By varying the pressure of the collection solvent, a range of reversible and reproducible flow-rates were obtained at both high (400 bar, 3.5 to 0.2 ml/min liquid CO_2) and low (100 bar, 0.8 to 0.1 ml/min CO_2) SFE pressures using a 50 μm I.D. capillary restrictor. At low (0.2 ml/min) SF flow-rates the solubility of several metal complexes (e.g., ferrocene and Ni[$C_{22}H_{22}N_4$] complex) was measured and reproducible solubility values and flow-rates (R.S.D.<8%) were obtained.

Keywords: Flow control/collection device; Supercritical fluid extraction; Capillary restrictor; Alkanes

1. Introduction

Supercritical fluid extraction (SFE) is rapidly becoming an established alternative to conventional solvent extraction, as supercritical fluids have been shown to be able to extract a wide range of analytes from real world samples. Using SFE, the supercritical fluid (SF) is passed through the sample matrix and the analytes of interest are systematically extracted into the bulk fluid. The solvated analytes are then swept from the extraction cell to the collection device, where the SF is depressurized to a gas and the analytes are collected. To simultaneously maintain the extraction pressure in the SFE system and depressurize the SF in a controlled manner, an electric feedback regulator [1–3], a manual backpressure regulator or a capillary restrictor [4,5] is required.

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To collect the analytes, several off-line collection methods are available, including recovery of the extract into an empty collection vial [6], a pressurized [7,8] or atmospheric [4,5,9] organic solvent, or collection onto a sorbent resin [10,11] and/or cryogenically cooled surface [12]. On-line collection methods have also been used, where the extraction system is directly coupled to various chromatographic techniques such as gas chromatography (GC) [13,14], high-performance liquid chromatography (HPLC) [15], supercritical fluid chromatography (SFC) [16,17], gel permeation chromatography (GPC) [18] and thin layer chromatography (TLC) [19].

A problem arises in that the procedures taken to increase the collection efficiency of the system often cause restrictor plugging problems, and the steps taken to eliminate restrictor plugging may result in poor collection efficiencies. For example, one of the simplest and most efficient ways to recover the target analytes is to slowly (e.g., <2 ml/min CO_2 as measured at the pump head) depressurize the extract directly into the collection solvent using a small internal diameter (typically ≤50 μm I.D.) capillary restrictor [4,20]. However, as the extract is depressurized inside the restrictor, this results in a decrease in the solubility of the analytes in the SF passing through the capillary. This reduction in analyte solubility, combined with the Joule–Thomson cooling effect of the expanding extraction fluid at the restrictor exit, can lead to analyte precipitation and restrictor plugging. In an attempt to maintain a constant extraction flow-rate, the restrictor [4,21] and/or collection device [4,22] have been heated but these approaches can result in poor analyte recoveries and an increase in solvent evaporation [4,20].

To overcome the problems of maintaining both a constant flow-rate and good collection efficiency, previous attempts have employed a two-step collection process [10,23]. In the initial step the extraction flow-rate is maintained using a heated electric feedback regulator that deposits the extracted analytes onto a cooled inert surface or sorbent resin (the regulator being unsuitable for direct analyte collection in an organic solvent). In the second step at the end of the extraction, the retained analytes are flushed from the analyte trap with a few ml of organic solvent. While this method has proved an ideal means of analysing real world samples, the apparatus required is complicated, expensive and involves an additional sample handling step.

In this study a SF flow control/collection device has been developed which can simultaneously maintain the extraction flow-rate and collect the extract directly in an organic solvent [24]. With this device, the extract is partially depressurized through a heated capillary restrictor and into a pressurized collection solvent, so that both temperature and pressure are used to maintain the solubility of the extract in the SF. The pressurized mixture is finally depressurized to atmospheric conditions using a backpressure regulator, so the extract can be recovered in the collection vial. The collection efficiency of the device was determined by spiking a mixture of *n*-alkanes onto Tenax-TA and performing SFE over a range of flow-rates. The ability of the design to eliminate restrictor plugging was assessed by monitoring the SFE flow-rate of several real world samples and measuring the solubility of an analyte in the SF.

2. Experimental

2.1. Standards and samples

The collection efficiency of the apparatus was assessed using a neat mixture of nine *n*-alkanes (C_6, C_7, C_8, C_9, C_{10}, C_{12}, C_{14}, C_{16} and C_{18}) obtained from Aldrich (Gillingham, UK). Two real world samples with differing physical and chemical properties were chosen to test the ability of the supercritical apparatus to maintain a continuous extraction flow-rate. The samples included a plant material (e.g., lavender) obtained from a local cosmetic store and an environmental sample (e.g., a highly contaminated petroleum waste sludge). The ability of the apparatus to measure the solubility of an analyte was also assessed using two metal complexes, namely ferrocene (Aldrich) and a nickel complex, 5,7,12,14-tetramethyl-2,3:9,10-dibenzo[*b,i*][1,4,8,11] tetraazacyclotetradecine nickel (II) ($Ni[C_{22}H_{22}N_4]$), synthesized at British Nuclear Fuels plc.

2.2. Supercritical fluid apparatus

Supercritical fluid extraction and solubility measurements were performed using two ISCO Model 260D syringe pumps (ISCO, Lincoln, NE, USA), one containing industrial (CP) grade carbon dioxide (BOC Ltd, London, UK) and the other containing HPLC grade methanol (Fisons, Loughborough, UK). The extraction cell and pre-equilibration coil (1 m× 0.76 mm I.D.×1.6 mm [1/16 inch] O.D. coiled stainless steel tubing placed before the extraction cell to pre-warm the CO_2 to the extraction temperature) were maintained at 60°C using a Carlo Erba Fractovap gas chromatograph oven. Collection efficiency studies were performed using a 0.5-ml extraction cell (Phase Separations, Clwyd, UK), flow-rate studies were performed using a 3.5-ml extraction cell (Keystone Scientific, Bellefonte, PA, USA) and solubility studies were performed using a 10-ml extraction cell (Keystone Scientific). The extraction cells were connected to the SFE system using "slip-free" finger tight connectors (Keystone Scientific). The flow-rate of the SF through the extraction cell was controlled by an 11-cm-long, 50 μm I.D. stainless steel restrictor (Coopers Needle Works, Birmingham, UK) situated inside the detector heater of the Carlo Erba Fractovap gas chromatograph (allowing the entire restrictor to be heated to up to 400°C). The outlet of the stainless steel capillary restrictor was connected to a 1/16″ (1.6 mm) stainless steel T-piece into which was pumped the pressurized collection solvent. The collection solvent was delivered from a Merck-Hitachi LC660 pump (Merck-Hitachi, UK) at a constant flow-rate. The organic solvent could be heated prior to entering the T-piece by passing the solvent through a stainless steel coil wrapped around a thermostatically controlled aluminium heating block.

The pressure of the collection solvent was maintained using either a Rheodyne 7037 back pressure regulator (Rheodyne, Cotati, CA, USA) or a GO-66 backpressure regulator (GO Inc., San Dimas, CA, USA). The Rheodyne regulator with a small, 100 μl, dead volume was ideally suited for the rapid recovery of the spiked analytes in the collection efficiency study, whereas the GO-66 with a large, 7 ml, dead volume was required for the solubility study as the pressure could be finely adjusted. For the collection efficiency study the 1/16″ (1.6 mm O.D.) stainless steel exit tube of the Rheodyne regulator was (if needed) thermostatically heated by wrapping flexible heating tape around the tube and insulating the heating element with heat shrink tubing (RS, Birmingham, UK). The heating system was regulated with a J-type thermocouple and a temperature controller unit (Model 6102; Omega, Stamford, CT, USA). For the solubility study, the GO regulator was heated by placing the device on a thermostatically controlled hot plate. The extract and collection solvent (depressurized through the back-pressure regulators) were collected in a 20-ml glass vial which was either initially empty (solubility study) or contained 6 ml of dichloromethane (collection efficiency study). If the exit tube on the back-pressure regulator was heated the vial was cooled to ca. −10°C in a bath of acetone and ice.

2.3. Methodology

The collection efficiency of the apparatus was determined by filling a 0.5-ml extraction cell with ca. 400 mg of 60–80 mesh (250–180 μm O.D.) Tenax-TA and spiking 5 μl of the neat hydrocarbon mixture (ca. 555 μg of each analyte) into the middle of the sorbent resin. The cell was then immediately sealed, to prevent loss of any volatile components, and was connected to the SFE apparatus. The sorbent resin was extracted for 10 min with CO_2 at 400 bar and 60°C. At the end of the extraction, the collection solvent was spiked with the internal standard *n*-pentadecane and analysed using capillary GC.

To assess the ability of the apparatus to maintain a continuous extraction flow-rate, real world samples were extracted as received using a 3-g sample size. However, the petroleum waste sludge sample did require a bed of 100 μm O.D. silanized glass beads (Alltech, Carnforth, UK) to be placed between the sample and the 0.5 μm extraction cell outlet frit, to prevent the frit (not the restrictor) from becoming plugged. The samples were extracted for 30 min using CO_2 at 400 bar and 60°C, and the corresponding SF flow-rate generated during the extraction was monitored as liquid CO_2 delivered from the pump.

The ability of the apparatus to measure the solubility of an analyte was also assessed by placing a 1:1 mixture (10 g) of silanized glass beads (100 μm O.D.) and a metal complex (e.g., ferrocene or $Ni[C_{22}H_{22}N_4]$) inside a 10-ml extraction cell and "solubilizing" the metal complex at various SF temperatures and pressures, then collecting the sample in methanol (ferrocene) or chloroform (nickel complex). The flow-rate of the CO_2 during the solubility study was monitored at the pump and the quantity of ferrocene and $Ni[C_{22}H_{22}N_4]$ complex recovered was determined from the UV–Vis spectroscopic analysis of the extracts at 439 nm and 394 nm, respectively.

To operate the SF system, a series of logical steps were followed. The apparatus was initially pressurized by pumping CO_2 at constant pressure through valve A on the two stem valve and through the capillary restrictor and backpressure regulator (BPR) (see Fig. 1). With CO_2 flowing through the backpressure regulator, the regulator was adjusted until the required flow-rate was obtained. The collection solvent was then pumped into the T-piece and mixed with the CO_2, so that a mixture of CO_2 and organic solvent was depressurized through the regulator. At this point, the backpressure regulator was readjusted to accommodate for the change in fluid viscosity. Once the flow-rate had been re-established (typically within 1 to 2 min), the SFE or solubility study commenced by opening valve B on the two stem valve to pressurize the extraction cell. Once pressurized, either valve B was closed, to undertake a static investigation, or the outlet valve of the cell was opened, to begin a dynamic extraction. Under dynamic extraction conditions, valve A was closed so that the SF passed solely through the extraction cell. Thus, during an extraction the SF passed through the cell, before partially depressurizing through the capillary restrictor into the pressurized collection solvent. Finally, the CO_2–organic solvent mixture was depressurized to atmospheric conditions in the collection vial using the backpressure regulator.

2.4. Gas chromatographic analysis

All GC analyses were performed with a Carlo Erba Fractovap gas chromatograph with flame ionisation detection using helium as the carrier gas. The injections were performed in the split mode with a 60:1 split ratio into a BP1 fused-silica capillary column (25 m×0.32 mm I.D., 0.5 μm film thickness). The injector and detector temperatures were maintained at 250°C. The oven temperature at in-

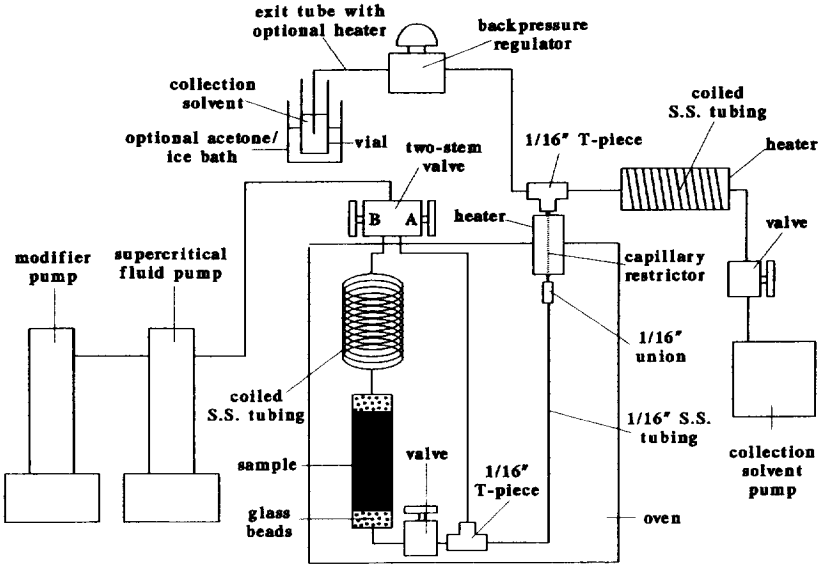

Fig. 1. Schematic diagram of variable flow control and collection device for use with supercritical fluids.

jection was 30°C for 3 min followed by a temperature ramp at 20°C/min to 250°C.

3. Results and discussion

3.1. Controlling the flow-rate of the supercritical fluid

All SFE systems require a flow control device which generally consists of either a simple capillary restrictor that directly depressurizes the extract into the collection solvent, or a more elaborate variable backpressure regulator which maintains a constant extraction flow-rate but is not amenable to direct organic solvent collection. The apparatus used in this study employs both a backpressure regulator and a capillary restrictor. The regulator controls the flow-rate of the extraction fluid and the pressure of the collection solvent, whereas the restrictor is used to ensure a one-way flow, by partially depressurizing the extract into the pressurized organic solvent. Using the backpressure regulator to set the pressure of the collection solvent, a wide range of SF flow-rates (e.g., 3.0 to 0.2 ml/min) can be obtained (Fig. 2). The SFE flow-rate is therefore determined by the pressure difference between the SF in the extraction cell and the collection solvent in the backpressure regulator (Fig. 1). By decreasing the pressure of the collection solvent, the extraction flow-rate is increased within seconds and vice-versa, so a range of flow-rates can be quickly achieved.

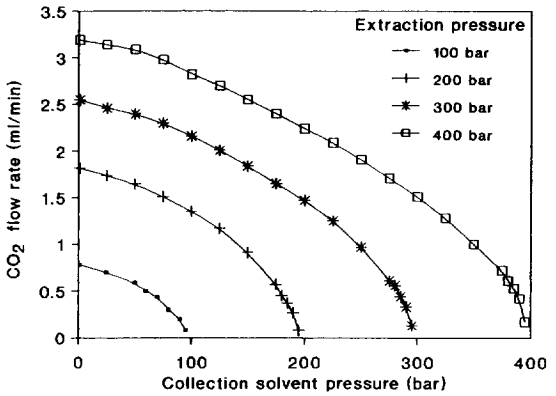

Fig. 2. Effect of collection solvent pressure on the supercritical fluid extraction flow-rate. All the flow-rates were achieved using a 50-μm I.D. stainless steel capillary and the collection solvent pressure was controlled by a GO-66 backpressure regulator.

With this new design, several extraction flow-rates could be obtained at a constant extraction pressure using the same 50 μm I.D. capillary restrictor. Thus, the apparatus eliminates the need to change the capillary I.D. to modify the flow-rate, as was previously required with the conventional use of the restrictor. At high SF pressures (e.g., 400 bar), both extraction (typical SF flow-rate 0.5–2.5 ml/min) and solubility (typical SF flow-rate 0.2–1.0 ml/min) studies can be carried out with this system. Acceptable flow-rates were also produced at the low (e.g., 100 bar) SF pressures. It should be note that the flow-rate could also be controlled by combining the SF and collection solvent at the same pressure without the use of a capillary restrictor. The extraction pressure and flow-rate would therefore be solely controlled by the backpressure regulator. However, the use of a capillary restrictor is desirable, as the initial partial depressurization of the SF into the collection solvent ensures that the CO_2 always flows into the organic solvent rather than the reverse procedure, so avoiding contamination of the sample matrix with organic solvent.

3.2. Analyte collection efficiency

Previous studies have shown that the restrictor or flow control device normally needs to be heated during an extraction, to ensure that a stable and reproducible SF flow-rate is obtained. Thus, the effect of the restrictor heater and the backpressure regulator temperature on the collection efficiency of the novel apparatus was investigated and the recoveries compared to those achieved using a conventional capillary restrictor, where the analytes are depressurized directly into an organic solvent at atmospheric pressure. Table 1 shows that with a conventional, unheated capillary restrictor, analytes as volatile as *n*-octane can be efficiently (>90%) collected. However, when the restrictor is heated (e.g., with a heat gun), the collection efficiency of the system significantly decreases, as *n*-decane becomes the most volatile analyte that can be quantitatively recovered. The problem with heating the conventional restrictor is that it indirectly heats up the collection

Table 1
Effect of restrictor heater temperature on the collection efficiency of *n*-alkanes using various collection designs

n-Alkane		Percent collected (%)[a]							
		Conventional collection[b]		Restrictor heated[c]			Restrictor and BPR heated[d]		
		Not heated	Heated	20°C	100°C	300°C	20°C	100°C	300°C
Hexane	C_6	38 (8)	21 (18)	55 (12)	34 (9)	32 (11)	36 (16)	40 (10)	50 (13)
Heptane	C_7	78 (2)	54 (18)	87 (5)	84 (5)	79 (4)	79 (5)	77 (4)	76 (1)
Octane	C_8	91 (2)	79 (8)	98 (4)	93 (5)	91 (3)	96 (5)	94 (5)	91 (1)
Nonane	C_9	95 (3)	88 (7)	102 (3)	97 (4)	96 (3)	101 (7)	100 (5)	96 (1)
Decane	C_{10}	99 (1)	97 (4)	103 (3)	99 (3)	98 (2)	102 (6)	102 (5)	98 (1)
Dodecane	C_{12}	98 (1)	96 (4)	102 (2)	100 (2)	97 (2)	103 (5)	103 (5)	99 (2)
Tetradecane	C_{14}	99 (2)	97 (3)	101 (2)	100 (1)	98 (1)	102 (5)	102 (5)	99 (1)
Hexadecane	C_{16}	98 (1)	97 (2)	99 (2)	100 (2)	98 (1)	103 (6)	102 (5)	100 (1)
Octadecane	C_{18}	100 (2)	99 (2)	98 (4)	102 (1)	97 (2)	102 (5)	102 (5)	101 (1)

[a] Value in parentheses is the relative standard deviation (%) of triplicate 10-min extractions using 400 bar, 60°C, CO_2 at 1 ml/min with dichloromethane as the collection solvent.

[b] The 400 bar, 60°C SF CO_2 extract was depressurized into dichloromethane at atmospheric pressure using a 30 μm I.D. fused-silica capillary restrictor. When the restrictor was heated, a heat gun was used. During the extraction, dichloromethane was manually added to the collection vial at ca. 0.4 ml/min to maintain the collection solvent volume.

[c] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using an unheated or thermostatically heated (e.g., 100°C and 300°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent were then completely depressurized into the collection vial using a backpressure regulator (BPR). During the extraction dichloromethane was automatically added to the collection vial at 0.45 ml/min to maintain the collection solvent volume.

[d] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using an unheated or thermostatically heated (e.g., 100°C and 300°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent was then completely depressurized through a heated (+20°C) backpressure regulator (BPR) and into a cooled (−10°C) collection vial. During the extraction, dichloromethane was automatically added to the collection vial at 0.35 ml/min to maintain the collection solvent volume.

solvent, so the volatile analytes are inefficiently retained in the poorly cooled solvent.

With this new collection design the entire length of the capillary restrictor is heated without significantly warming the collection solvent, as fresh organic solvent is continually pumped through the T-piece where the extract exits the restrictor and enters the collection solvent system (Fig. 1). If the solvent is slightly warmed it quickly cools as it is pumped to the backpressure regulator and is further cooled to ca. −5°C as the extract is depressurized to atmospheric conditions in the collection vial. It is therefore possible to use very high restrictor heater temperatures of 300°C and yet get the same recoveries as those achieved using a conventional unheated restrictor, namely by quantitatively trapping analytes as volatile as *n*-octane (see Table 1).

For real world samples it is sometimes necessarily to heat the restrictor and the backpressure regulator in the new design (as discussed later). Heating both flow control devices significantly increases the rate of solvent evaporation in the collection vial and reduces the adiabatic cooling of the organic solvent. Thus, during the extraction, the solvent in the vial is typically only cooled to ca. 6°C. To reduce the solvent evaporation and enhance analyte recoveries, the collection vial was externally cooled to −10°C with an acetone–ice bath. Using the cooled vial with the heated restrictor and backpressure regulator, analytes as volatile as *n*-octane were efficiently collected and the recoveries were comparable to those obtained with the other collection methods (see Table 1).

One of the major disadvantages of using a conventional capillary restrictor is that CO_2 rapidly depressurizes in the atmospheric collection solvent, so there is poor analyte–solvent contact and hence poor collection efficiencies. The new collection method has the advantage that the analytes are introduced into the collection solvent under pressure, so there is significant analyte–solvent contact prior to recovery of the extract in the collection vial. Consequently, when the CO_2–collection solvent mixture is depressurized to atmospheric conditions using the back-

pressure regulator, the presence of the organic solvent simultaneously depressurizing with the CO_2 enhances the collection efficiency of the system, as the organic solvent forms sizeable liquid droplets which contain or scavenge the extracted analytes. A similar phenomenon has been observed with the use of modifiers in SFE [12]. Indeed, (as discussed later in Section 3.4, when the collection solvent was pumped at a sufficiently high flow-rate (e.g., 1 ml/min) no collection solvent was initially required in the collection vial, as the analytes were efficiently trapped solely in the organic solvent depressurizing from the backpressure regulator.

With this novel apparatus, the collection solvent flow-rate is independent of the extraction conditions, as the SF is operated at constant pressure and the organic solvent at constant flow. Therefore, by adjusting the collection solvent flow-rate to match the solvent evaporation rate in the collection vial, a constant solvent volume can be maintained in the vial throughout the extraction procedure. This automatic addition of solvent is more convenient and less labour intensive than the manual additions required with conventional SFE methods. Typically, a 0.45-ml/min solvent flow-rate is required to maintain the collection solvent volume and this is comparable to the solvent usage incurred when heating a conventional capillary restrictor with a heat gun (See Table 1). If the collection vial in the new design is cooled (as is the case when the backpressure regulator is heated), then only 0.35 ml/min solvent addition is required, which in a typical 30 min extraction results in ca. 16 ml of organic solvent being used (this includes the original 6 ml of solvent present in the vial at the beginning of the extraction).

The results in Table 1 were obtained using a 1 ml/min SFE flow-rate which is an adequate flow-rate for a number of real world samples, but is often inadequate for large samples (e.g., >10g) or samples with solubility limitations. For such flow-dependent samples, a higher extraction flow-rate of 2.5 ml/min has proved beneficial and the effects of this increased flow-rate on the collection efficiency of the system is shown in Table 2. Increasing the flow-rate through a conventional restrictor resulted in a significant decrease in the recovery of the volatile C_6 to C_8

Table 2
Effect of extraction flow-rate on the collection efficiency of *n*-alkanes using various collection designs

n-Alkane		Percent collected (%)[a]					
		Conventional collection[b]		Restrictor heated[c]		Restrictor and BPR heated[d]	
		1 ml/min	2.5 ml/min	1 ml/min	2.5 ml/min	1 ml/min	2.5 ml/min
Hexane	C_6	38 (8)	15 (37)	38 (5)	45 (7)	40 (10)	34 (9)
Heptane	C_7	78 (2)	69 (11)	78 (9)	77 (6)	77 (4)	77 (9)
Octane	C_8	91 (1)	84 (7)	93 (5)	92 (6)	94 (5)	93 (5)
Nonane	C_9	95 (1)	95 (2)	100 (3)	98 (4)	100 (5)	97 (4)
Decane	C_{10}	98 (1)	99 (2)	102 (2)	100 (3)	102 (5)	99 (3)
Dodecane	C_{12}	98 (1)	98 (1)	103 (1)	100 (2)	103 (5)	100 (2)
Tetradecane	C_{14}	99 (2)	100 (2)	102 (1)	101 (2)	102 (5)	100 (1)
Hexadecane	C_{16}	98 (1)	99 (1)	101 (2)	100 (3)	102 (5)	100 (1)
Octadecane	C_{18}	100 (2)	100 (1)	101 (1)	101 (5)	102 (5)	102 (1)

[a] Value in parentheses is the relative standard deviation (%) of triplicate 10-min extractions using 400 bar, 60°C, CO_2 at several flow-rates. Dichloromethane was used as the collection solvent.

[b] The 400 bar, 60°C SF CO_2 extract was depressurized into dichloromethane at atmospheric pressure using an unheated 30 μm I.D. fused-silica capillary restrictor. During the extraction, dichloromethane was manually added to the collection vial at ca. 0.4 ml/min to maintain the collection solvent volume.

[c] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using a thermostatically heated (100°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent was then completely depressurized into the collection vial using a backpressure regulator (BPR). During the extraction dichloromethane was automatically added to the collection vial at 0.45 ml/min to maintain the collection solvent volume.

[d] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using a thermostatically heated (100°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent was then completely depressurized through a heated (+20°C) backpressure regulator (BPR) and into a cooled (−10°C) collection vial. During the extraction dichloromethane was automatically added to the collection vial at 0.35 ml/min to maintain the collection solvent volume.

n-alkanes, as the analyte–solvent contact time is decreased and analyte aerosol formation increased. With the new collection method, the recoveries were independent of the extraction flow-rate, suggesting that the analytes had sufficient solvent contact at both the 1.0 and 2.5 ml/min SFE flow-rates.

Even when the extracted analytes have been collected in the organic solvent there is still the potential for analyte loss, because as the extraction continues the solvated analytes can be purged from the solvent in the collection vial. Table 3 shows the extent of purging using the various collection methods at several extraction times. When the collection solvent was significantly cooled, as occurred with the conventional capillary restrictor and the novel collection method with the collection vial placed in an acetone–ice bath (e.g., collection solvent temperatures of −30°C and −10°C, respectively), there was no detectable purging of the analytes with time. However, when the novel device was used with no external cooling of the collection vial, significant purging of the volatile analytes was observed, as analytes such as *n*-octane which were initially collected within the first 10 min of the extraction were partially purged for the solvent after 30 min of SFE. These analyte losses were due to the poor cooling of the collection solvent (e.g., ca. −5°C) during the extraction, as the depressurization of the SF occurred in the backpressure regulator rather than in the collection solvent. Therefore, if long extraction times are undertaken with the new design, an externally cooled collection vial may be required to ensure that the extracted analytes are efficiently retained in the collection solvent.

3.3. Constant extraction flow-rates with real world samples

Two real world samples; a highly contaminated environmental sample (e.g., a petroleum waste sludge) and a natural product (e.g., dried lavender) were selected to assess the ability of the novel

Table 3
Effect of extraction time on the collection efficiency of *n*-alkanes using various collection designs

n-Alkane		Percent collected (%)[a]					
		Conventional collection[b]		Restrictor heated[c]		Restrictor and BPR heated[d]	
		10 min	30 min	10 min	30 min	10 min	30 min
Hexane	C_6	38 (8)	37 (7)	38 (5)	20 (37)	40 (10)	36 (22)
Heptane	C_7	78 (2)	79 (9)	78 (9)	65 (15)	77 (4)	75 (8)
Octane	C_8	91 (1)	92 (5)	93 (5)	87 (8)	94 (5)	90 (2)
Nonane	C_9	95 (1)	98 (3)	100 (3)	95 (2)	100 (5)	95 (1)
Decane	C_{10}	98 (1)	99 (2)	102 (2)	97 (2)	102 (5)	99 (2)
Dodecane	C_{12}	98 (1)	100 (1)	103 (1)	96 (1)	103 (5)	100 (1)
Tetradecane	C_{14}	99 (2)	100 (2)	102 (1)	97 (1)	102 (5)	102 (2)
Hexadecane	C_{16}	98 (1)	101 (1)	101 (2)	97 (2)	102 (5)	101 (2)
Octadecane	C_{18}	100 (2)	100 (1)	101 (1)	99 (1)	102 (5)	102 (1)

[a] Value in parentheses is the relative standard deviation (%) of triplicate 10 and 30 min extractions using 400 bar, 60°C, CO_2 at 1 ml/min with dichloromethane as the collection solvent.

[b] The 400 bar, 60°C SF CO_2 extract was depressurized into dichloromethane at atmospheric pressure using an unheated 30 μm I.D. fused-silica capillary restrictor. During the extraction, dichloromethane was manually added to the collection vial at ca. 0.4 ml/min to maintain the collection solvent volume.

[c] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using a thermostatically heated (100°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent were then completely depressurized into the collection vial using a backpressure regulator (BPR). During the extraction, dichloromethane was automatically added to the collection vial at 0.45 ml/min to maintain the collection solvent volume.

[d] The 400 bar, 60°C SF CO_2 extract was partially depressurized into a pressurized (350 bar, 20°C) collection solvent (dichloromethane) using a thermostatically heated (100°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent were then completely depressurized through a heated (+20°C) backpressure regulator (BPR) and into a cooled (−10°C) collection vial. During the extraction, dichloromethane was automatically added to the collection vial at 0.35 ml/min to maintain the collection solvent volume.

apparatus to eliminate restrictor plugging. To rigorously test the design, a 3-g sample size was used and the samples were extracted in their native state. Fig. 3 shows the flow-rates obtained for the samples using both a conventional capillary restrictor and the new collection method. For the lavender sample (3 wt% extractable material) the use of a conventional capillary restrictor generated an erratic flow-rate, the restrictor requiring intermittent heating and after 15 min the flow had decreased by 50%. When the novel apparatus was used to extract the lavender, a stable and continuous extraction flow-rate was obtained throughout the extraction, although the restrictor needed to be heated to 200°C.

For the petroleum waste sludge sample, the use of a conventional capillary restrictor proved totally unsuitable, as the restrictor became irreversibly plugged within minutes of commencing the extraction and no amount of heating with the heat gun could re-establish the flow (see Fig. 3). Using the new collection method, a continuous extraction flow-rate was achievable, though there was an initial decrease in the flow-rate at the beginning of the extraction. By readjusting the backpressure regulator, the flow was quickly re-established and no further adjustments were required. To maintain the extraction flow-rate of the sludge, both the capillary restrictor (e.g., 200°C) and the exit tube on the backpressure regulator (e.g., 20°C) were heated. If the exit tube was not heated, plugging would occur and this appeared to be related to the high percentage of water (20 wt%) in the sample. Extracted water can easily pass through a heated capillary restrictor, but it freezes and plugs in the cooled backpressure regulator where final depressurization of the extract occurs.

Several other samples were investigated, including an ionic detergent, a metal complex, a polymer, air particulate matter and a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. Regardless of the analyte composition, if the sample contained a significant amount of water (>5 wt%), a heated restrictor and backpressure regulator were required to maintain the flow-rate, and when the sample had a low water content, only a heated capillary restrictor was needed. An alternative method is to mix the wet sample with a drying agent so as to retain the water in the extraction cell, but this approach requires an additional sample preparation step.

By analysing the real world samples, several further observations were noted concerning the new design. For example, if the restrictor became plugged, it could be quickly unplugged by back-flushing the capillary with pressurized collection solvent (see

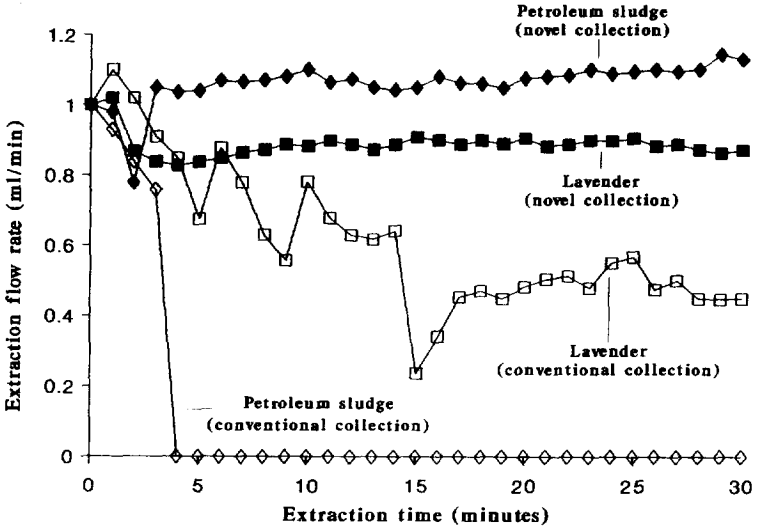

Fig. 3. Extraction flow-rate of real world samples obtained by using a conventional capillary restrictor and the novel collection method. To maintain the flow of the samples using the novel design, either a heated restrictor (e.g., lavender plant) or heated restrictor and heated backpressure regulator exit tube (e.g., petroleum waste sludge) were required.

Fig. 1). Using this technique, the down time of the equipment is kept to a minimum and the lengthy procedure of replacing the restrictor is avoided. The apparatus also has a two-way valve (shown as A B in Fig. 1) so CO_2 can bypass the extraction cell and clean the collection tubing and backpressure regulator between extractions, so avoiding the possibility of "carryover" between samples.

3.4. Solubility studies

SF solubility studies are often complicated by the saturated fluid causing severe plugging problems. Thus, a variety of methods have been used for determining solubility, including gravimetric [25,26], spectroscopic [27], chromatographic [28] and on-line procedures using several chromatographic detectors [29,30]. The simplest and most popular method is to pass the SF through a saturator cell containing the analyte, the resulting analyte-saturated SF is then pumped to a heated pressure reduction valve where it is depressurized and the analytes collected in a cooled U-tube. This flow-through system enables the recovered analytes to be analysed by a range of spectroscopic or chromatographic techniques. However, the system is not suitable for the investigation of volatile analytes.

The new device is a suitable flow-through system which can be used to measure the solubility of both semi-volatile and volatile analytes. Solubility measurements were made with the apparatus using both a highly soluble (e.g., ferrocene) and a poorly soluble (e.g., $Ni[C_{22}H_{22}N_4]$) metal complex. To ensure that the SF became saturated with the metal complex, a large, 8 g, sample size was used, and to establish a thermodynamic equilibrium, the flow-rate of the SF was reduced until there was no measurable effect on solubility. Equilibration was achieved with a supercritical flow of ca. 0.75 ml/min, though for the study a flow of 0.2 ml/min was used. Table 4 shows the solubility results obtained using the novel device. The reproducibility of the solubility measurements for both the ferrocene and nickel complexes were within 7% and this included all the possible errors associated with the extraction, collection and analysis of the samples. The reliability of the system was also reflected in the flow-rate values, which had similar relative standard deviations of less than 8%. Consistent flow-rates were achievable even at the high supercritical pressures where the CO_2 was saturated with up to 2 wt% ferrocene. A modifier (10 wt% methanol) was required to enhance the solubility of the nickel complex (Table 4). The presence of the modifier did not cause any problems for the device, as the flow-rate and solubility values obtained were as reproducible as those achieved with pure CO_2.

Using the novel apparatus, the solubility temperature, pressure and flow-rate conditions could be quickly varied during the analysis, so a large number of measurements could be sequentially undertaken

Table 4
Solubility of metal complexes in supercritical CO_2 using a novel apparatus

Analyte	CO_2 Pressure (bar)	CO_2 Flow-rate (ml/min)	Solubility (mole fraction)
Ferrocene[a]	134	0.21 (1.8%)	1.08×10^{-3} (5.7%)
(60°C CO_2)	245	0.20 (4.4%)	3.92×10^{-3} (6.2%)
	335	0.19 (7.8%)	4.83×10^{-3} (5.2%)
$Ni[C_{22}H_{22}N_4]$[b]	161	0.22 (3.1%)	6.72×10^{-7} (2.0%)
(60°C CO_2–10% MeOH)	252	0.20 (2.1%)	2.26×10^{-6} (6.7%)
	342	0.21 (2.6%)	3.05×10^{-6} (4.3%)

Values in parenthesis are relative standard deviations (%) based on triplicate determinations. The samples were partially depressurized into a heated (60°C) pressurized collection solvent using a thermostatically heated (300°C for ferrocene and 125°C for the nickel complex) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent were then completely depressurized into a collection vial using a heated (40°C) backpressure regulator.

[a] Collection solvent, 1 ml/min methanol.

[b] Collection solvent, 1 ml/min chloroform.

over a relatively short period of time. As the "extracts" were analysed by UV–Vis spectroscopic analysis, a detectable amount of sample could easily be collected within a 15-min time period, whereas gravimetric sampling could require several hours to collect a sufficient mass of sample to be weighed. It was therefore possible to generate all the ferrocene results presented in Table 4 within one working day. A full analysis of the solubility of the metal complexes using the novel collection method has been presented in a separate publication [31] and the solubility of several other samples, including an ionic detergent, an azo dye and a PAH have also been successfully obtained.

To maintain the SF flow-rate during the solubility study, the capillary restrictor was heated to either 125°C (nickel complex) or 300°C (ferrocene) and the backpressure regulator to 40°C. To ensure that the metal complexes were sufficiently solvated in the collection solvent, a large volume of organic solvent was used (e.g., 1 ml/min) and the solvent warmed (e.g., 60°C) prior to coming into contact with the SF (see Fig. 1). Despite the use of relatively high restrictor heater temperatures and a warm collection solvent (e.g., +15°C in the collection vial), the apparatus could still quantitatively recover the sample. For example, as shown in Table 5, by extracting the *n*-alkane test mix under identical conditions as those used in the solubility study, analytes as volatile as *n*-octane could be efficiently collected. These quantitative recoveries were related to the high collection solvent flow-rate which ensured that there was sufficient solvent to trap the sample during depressurization into the collection vial. Unfortunately, a high collection solvent flow-rate means that for a 30-min sampling, ca. 28 ml of organic solvent is accumulated in the collection device (the vial being initially empty at the start of the analysis). It is acknowledged that this would not necessarily be the method of choice for a SFE procedure.

Table 5
Collection efficiency of *n*-alkanes using the solubility apparatus

n-Alkane		Percent collected (%)[a]
Hexane	C_6	43 (27)
Heptane	C_7	75 (7)
Octane	C_8	91 (5)
Nonane	C_9	97 (4)
Decane	C_{10}	99 (4)
Dodecane	C_{12}	100 (3)
Tetradecane	C_{14}	100 (2)
Hexadecane	C_{16}	100 (1)
Octadecane	C_{18}	100 (2)

[a] Value in parentheses is the relative standard deviation (%) of triplicate 15-min extractions with 400 bar, 60°C CO_2 at 0.5 ml/min. The extract was partially depressurized into a pressurized (380 bar), heated (60°C) collection solvent (dichloromethane at 1 ml/min) using a heated (300°C) 50 μm I.D. stainless steel restrictor. The pressurized extract and collection solvent were then completely depressurized into the collection vial using a heated (40°C) backpressure regulator. The collection solvent temperature during extraction was ca. +15°C.

4. Conclusions

The collection apparatus used in this study was able to maintain a continuous extraction flow-rate and efficiently collect the extracts by using a heated capillary restrictor in conjunction with a backpressure regulator. The success of the method was due to the design using both temperature (e.g., heated restrictor) and pressure (e.g., pressurized organic solvent) to ensure that the extracted analytes remained solvated throughout the entire SFE apparatus. By using a backpressure regulator, the SF flow-rate could be set independently of the extraction pressure, so that both SFE (high flow-rate) and solubility (low flow-rate) studies could be undertaken. It was therefore possible to analyse several real world samples which had previously caused severe restrictor plugging problems.

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