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Rapid sequential separation of sedimentary lipid biomarkers via selective accelerated solvent extraction

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ABSTRACT

Accelerated solvent extraction (ASE) can be adapted for selective and sequential separation of saturated hydrocarbon, unsaturated/aromatic hydrocarbon and polar lipid fractions from complex organic extracts in environmental matrices. ASE extraction cells were packed with a layer of Ag^+ impregnated and activated silica gel, followed by an aliquot of the total lipid extract, and topped with another layer of activated silica gel. A fraction containing saturated hydrocarbons (F_{SAT}) was eluted with hexane. Then, cells were inverted for reversed solvent flow and compounds of increasing polarity were eluted with solvent of increasing eluotropic strength to yield an unsaturated/aromatic hydrocarbon fraction (F_{ARO}) separated from a polar lipid fraction (F_{POL}). Gas chromatography–mass spectrometry analysis demonstrated high recovery of standards in F_{SAT} (90 ± 3%), F_{ARO} (82 ± 4%) and F_{POL} (87 ± 3%). Average compound recovery and efficiency of separation between lipid fractions were significantly improved ($p < 0.05$) relative to separation with gravity column chromatography using similar stationary phases. Overall, this selective extraction method affords reliable, semi-automated separation of compound classes in total lipid extracts according to saturation and polarity and is well suited for molecular characterization and compound-specific isotopic analysis.

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1. Introduction

Accelerated solvent extraction (ASE) is a well established technique for exhaustive lipid extraction of diverse environmental matrices (Carabias-Martínez et al., 2005; Ridgway et al., 2007; Sun et al., 2012), including sediments and biological tissue. Recent studies have demonstrated its capability for preparative (*in-cell*) retention of matrix interferences during selective lipid extraction, via selective ASE techniques (Björklund et al., 2006; Pörschmann and Carlson, 2006; Pena et al., 2010; Haskins et al., 2011; Zhang et al., 2012; Choi et al., 2014). In a practical sense, selective extraction incorporates chromatographic adsorbents into conventional ASE procedures in order to simultaneously extract and isolate (*clean up*) selected lipids from incidental organic components.

Recent developments in selective extraction methods using ASE have generally focused on environmental and industrial applications (Schantz, 2006; Zuloaga et al., 2012; Gilart et al., 2014), particularly high throughput screening of organic pollutants in soils and sediments. For example, Ong et al. (2003) describe an ASE method for rapid isolation of polycyclic aromatic hydrocarbons (PAHs) from industrial soils. Similarly, Choi et al. (2014) describe an ASE method for the isolation of PAHs from marine sediments. In both these methods, as well as others (Canosa et al., 2007; Hussen et al., 2007; Ghosh et al., 2011; Osman and Saim, 2013; Net et al., 2014), sediments were ultimately loaded into extraction cells packed with one adsorbent (e.g. silica gel) and then eluted with one solvent [e.g. hexane–dichloromethane (DCM; 85:15 v:v)] to isolate a single class of compounds, such as unsaturated hydrocarbons.

Many paleoenvironmental and energy related organic geochemical studies require separation of complex extracts into more discrete compound fractions prior to molecular and isotopic characterization. Although recent studies using ASE have incorporated multiple adsorbents (Muijs and Jonker, 2009; Pena et al., 2010; Zhang et al., 2012; Do et al., 2013) or multiple solvent sequences (Björklund et al., 2006; Lundstedt et al., 2006; Zuloaga et al., 2012), they focused on *exclusion* of undesirable compounds rather than on *separation* or *isolation* of desirable compounds (Gauchotte-Lindsay et al., 2014). These studies suggest that ASE selective separation has potential for isolating entire compound fractions from complex mixtures extracted from sediments and sedimentary rocks via a combination of both multiple stationary (adsorbent)

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and mobile (solvent) phases. Here, we describe a novel ASE method for quantitative semi-automatic separation into three strategic compound fractions – saturated hydrocarbons, unsaturated/aromatic hydrocarbons and polar lipids – from complex organic mixtures (e.g. environmental total lipid extracts). Using gas chromatography–mass spectrometry (GC–MS), we demonstrate that this separation method is robust for rapid chromatographic separation of complex organic mixtures into fractions suitable for molecular characterization and isotopic analysis.

2. Material

2.1. Reagents and lipid standards

Analytical grade *n*-hexane, DCM and MeOH were degassed by sparging with N_2 prior to use. Silica gel [Si-gel (60 Å; 70–230 mesh)], quartz sand (20–30 mesh) and glass microfiber filters (GF/C grade) were ashed for 8 h at 450 °C to remove potential contamination. Si-gel was activated for 2 h at 150 °C. Ag^+ impregnated Si-gel (5% by wt) was prepared according to Chakraborty and Raj (2007).

Lipid standards were used as a reference mixture composed of *n*-alkanes (C_{15} [*n*-C_{15}] through C_{22} and C_{31}); saturated hydrocarbons (pristane and 5α-cholestane); unsaturated hydrocarbons (eicos-1-ene and squalene); PAHs (fluoranthene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene); *n*-alcohols (C_{12} [*n*-C_{12}OH] and C_{28}); *n*-alkanoic acids (C_{16} [*n*-C_{16}OOH] and C_{26}, and octadecanedioic acid [*n*-C_{18}di-OOH]); benzene-1,2-dicarboxylic acid (phthalic acid); and 2,4-dihydroxybenzoic acid (α-resorcylic acid). The mixture was prepared at two different concentrations (1 or 100 ng/μl per standard) in DCM:MeOH (85:15 v:v).

2.2. Environmental (soil) sediment

Wetland sediment (A and B_h horizons) was collected from beneath patches of *Carex trisperma* that fringe Bear Meadows bog in central Pennsylvania (40.732°N, 77.754°W). Collected sediment was freeze-dried and homogenized using a mortar and pestle.

3. Methods

3.1. Lipid extraction and separation

Stock extraction cells (33 ml) were packed first with a microfiber filter (Section 2.1) followed by 1 g sand to prevent clogging (Pörschmann et al., 2001) and then loaded with sediment (10 g). Remaining dead volume was filled with sand. The reference mixture (50 μl) was loaded into cells in a similar manner with identical packing material. Packed and loaded cells were extracted with DCM:MeOH (85:15 v:v) with a 70% flush volume (Quénéa et al., 2012) in a Dionex ASE 200 system set to 3 cycles of 5 min at 100 °C and 10.3 MPa. The total lipid extract (TLE) was gently blown to dryness under N_2 (c.f. Denis et al., 2012) and reconstituted in 50 μl extraction solvent.

3.1.1. Compound separation: column construction

As above, stock extraction cells (33 ml) were packed with a filter and 1 g sand (Fig. 1). They were then packed with 2 g Ag^+ Si-gel, a second filter (to prevent mixing between layers), and 5.5 g activated Si-gel. A third filter was added atop these layers, to which reconstituted TLE in 50 μl DCM was added. Cells were then packed with another 1 g activated Si-gel. Remaining dead volume was filled with sand (Björklund et al., 2001; Hubert et al., 2001).

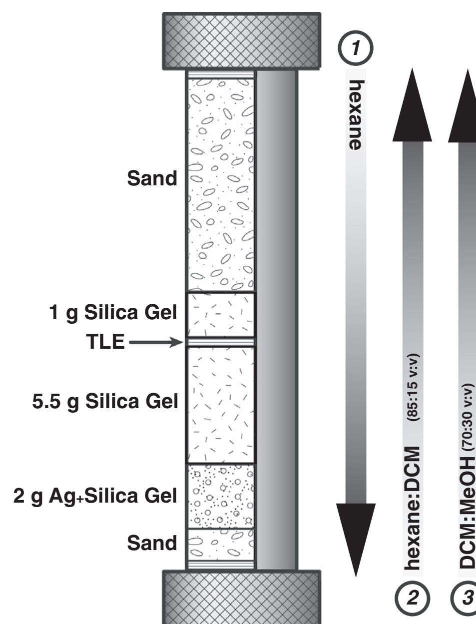

Fig. 1. Schematic representations for column construction and sequential elution as described in Section 3.2. Packed and loaded columns were first eluted with hexane. Then, they were inverted to reverse solvent flow and back elute with hexane:DCM (85:15 v:v) and DCM:MeOH (70:30 v:v), respectively.

3.1.2. Compound separation: solvent elution schedule

Following convention for gradient elution chromatography (Hirsch et al., 1972; Snyder et al., 1979; Radke et al., 1980; Jandera and Churácek, 1985), ASE columns were sequentially eluted with solvent of increasing eluotropic strength (ε^0; Meyer and Palamareva, 1993) to separate the TLE into fractions based on unsaturation and polarity. Less conventionally, ASE columns were inverted to reverse solvent flow and back elute more polar compounds. For this (Fig. 1), a saturated hydrocarbon fraction (F_{SAT}) was eluted with hexane (ε^0 0.01) because of its overall low retention on activated Si-gel ($k < 0.15$; Radke et al., 1980; Dark, 1982) with this solvent. Unsaturated hydrocarbons (e.g. eicos-1-ene) with similarly low retention on activated Si-gel ($0.15 < k < 1.15$; Radke et al., 1980) with hexane were rather retained on underlying Ag^+ Si-gel (McKay and Latham, 1980; Bennett and Larter, 2000; D'Andrea et al., 2007). Elution with hexane proceeded for 1 min at 50 °C and 3.4 MPa, with 30% (ca. 12 ml) total flush volume.

Next, the ASE separation column was inverted and back eluted with hexane:DCM (85:15 v:v; ε^0 0.13) and DCM:MeOH (70:30 v:v; ε^0 0.72) to collect unsaturated/aromatic hydrocarbon (F_{ARO}) and polar lipid (F_{POL}) fractions, respectively (Morris, 1966; Hirsch et al., 1972; Radke et al., 1984; Björklund et al., 2006; Lund et al., 2009). Back elution proceeded at 50 (F_{ARO}) or 70 °C (F_{POL}) and 3.4 MPa in 3 cycles of 1 min, each with 60% (ca. 20 ml) total flush volume.

3.2. Reference procedures

Gravity (*flash*) column chromatography served as a reference procedure (Still et al., 1978; Bastow et al., 2007) for comparison with the ASE method. Reconstituted TLE (Section 3.1) was loaded onto a pipette column packed with 2 g activated Si-gel (Bastow et al., 2007). Combined saturated and unsaturated/aromatic hydrocarbons were eluted with 8 ml hexane:DCM (85:15 v:v) and the polar lipid fraction (F_{POL}) was eluted with 8 ml MeOH. Then, combined hydrocarbons were loaded onto a second pipette column packed with 2 g Ag^+ Si-gel (Bennett and Larter, 2000) and eluted with 8 ml hexane and 8 ml DCM to collect F_{SAT} and F_{ARO}, respectively.

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