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Use of factorial design for the multivariate optimization of polypropylene membranes for the cleanup of environmental samples using the accelerated membrane-assisted cleanup approach

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ABSTRACT

Accelerated membrane-assisted cleanup (AMAC) is a recently developed method to purify extracts from matrix rich samples such as fish tissue and sediments. In this study, we tested the applicability of cast polypropylene (CPP) membranes in AMAC and evaluated the optimized dialysis procedure for the cleanup of extracts of fish tissue. Design of experiments was used to optimize the factors temperature, solvents and static time of dialysis. Main factors influencing dialysis procedure were solvents and temperature as well as the number of cycles. For the CPP membrane the optimal parameters were a temperature of 55 °C, a solvent mixture of *n*-hexane:acetone (90:10, v:v), a static time of dialysis of 6 min and 20 dialysis cycles. Comparing to the LDPE membrane this was a reduction of dialysis time from 160 to 120 min, but a higher solvent use of 150 ml per sample. However, compared to LDPE membranes CPP exhibited a lower retention of fish tissue matrix and thus reduced cleanup efficiency. Compound specific structural descriptors such as the molecular weight, the van der Waals volume and a shape factor were calculated to explain differences in diffusivity of the different model compounds. We concluded that the permeation of the molecules was related to molecular shapes and the availability of free solvent cavities in the membranes.

1. Introduction

Cleanup procedures are an inherent part of the workflow of bio- and chemical–analytical analysis of organic micro-pollutants in extracts of complex environmental sample matrices such as biological tissues, sediments, suspended particulate matter and soils. The separation of matrix compounds (e.g. lipids, humic acids, and pigments) is necessary to avoid their interferences with chemical analysis and bioassays as well as to achieve the requirements of high, replicable and reproducible analytes recoveries [1]. Established approaches for this purpose are size-exclusion methods like gel permeation chromatography (GPC), column chromatography using different sorbents (e.g. silica gel, alumina, Florisil®), and chemical treatment (saponification, oxidation) [1–5]. These methods are often optimized and selective for the analysis of specific target compounds. However, for chemical analysis in combination with bioanalytical approaches such as effect-directed analysis (EDA) and toxicity identification evaluation (TIE) [6] nonselective rather than compound specific cleanup procedures are recommended to recover as many potentially toxic compounds as possible [1,7].

Membrane-assisted cleanup techniques such as membrane dialysis extraction (MDE) [8], rapid dialysis procedure (RDP) [9] and accelerated membrane assisted cleanup (AMAC) [1] can help to bridge the gap between nonselectivity for different classes of small molecules and a significant retention of matrix macro molecules. AMAC – based on the RDP approach – is a cleanup method to purify lipid and organic matrix rich extracts of biota and sediment samples [1,10–15]. A further development of the method utilizes AMAC for the direct extraction and in-cell cleanup of extracts of sewage sludge with pressurized membrane-assisted liquid extraction (PMALE) [16]. Membranes are morphologically classified in microporous and nonporous ones referring to the presence or absence of pores in their structures [17]. The transport in microporous membranes is mostly influenced by viscous flow and sieving dependent on membrane pore characteristics (porous transport or flow model) and in nonporous membranes by molecular interactions of the permeating compound with the membrane material (solution-diffusion model) [17–19]. In the latter the transport of small molecules is a random and individual molecular motion influenced by the segment mobility of the polymer chains and the free

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solvent cavities of approximately 5–10 Å [19–21]. The driving force for the transport in membranes is a chemical potential by a gradient across the membrane [17,18], e.g., supported by a frequent renewal of the receiving or acceptor solvent phase [22,23]. Thus, the separation capability of the dialysis procedure depends on the nonselective transport of the small molecular analytes from a complex raw extract across the membrane with a significant or total retention of the matrix macro molecules due to size exclusion or slow diffusion referring in microporous and nonporous membranes, respectively.

In membrane-assisted cleanup methods or dialysis often commercially available low-density polyethylene (LDPE) tubes are used [1,8,16,24] because of easy handling, low costs, and stability in a variety of organic solvents [24]. As an alternative polypropylene membrane with a thickness of 0.03 mm and 0.05 mm have been applied in membrane-assisted solvent extraction (MASE) for the analysis of chlorophenols and triazines in water samples [25,26]. However, literature regarding the usage of polypropylene membranes for cleanup or dialysis purposes is limited.

Design of experiments (DOE) using the full factored central composite design (CCD) approach was used to optimize the recovery by varying the factors temperature, solvents and static time of dialysis. In contrast to univariate experiments where the factors are studied one by one, CCD considers all factors and factor levels at the same time [27,28]. CCD facilitates a reduction in the number of experiments with a complete coverage of the experimental space to be analyzed. It combines a core two-level factorial design (edge points) describing linear effects, a center point denoting the middle of all factor levels (or component ranges in a mixture) and axial star points representing quadratic effects resulting in an approximately spherical experimental space.

In our previous study we used LDPE membranes for the AMAC approach [1]. Thus, this paper evaluates the usability of polypropylene membranes for AMAC. Two different polypropylene membranes were studied and one was optimized for the parameters temperature, static time of dialysis, number of cycles and solvents. The optimized procedure was compared with the LDPE procedure for lipid removal efficiency.

2. Material and methods

2.1. Chemicals

Target analytes selected to develop AMAC represented different compound classes and physicochemical properties are listed in Table S1 (Supplementary data). All standards were purchased from LCG Promochem (Wesel, Germany), Dr. Ehrenstorfer (Augsburg, Germany) or Sigma Aldrich (Steinheim, Germany). The solvents acetone, *n*-hexane, and toluene (Suprasolv® or LiChrosolv® grade) were obtained from Merck (Darmstadt, Germany).

2.2. Gas chromatography–mass spectrometry

GC–MS analyses were carried out on a HP 6890 GC coupled to a HP MSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film HP-17 MS or an HP-5 MS fused capillary silica column, a 5 m pre-column (Agilent J&W, Folsom, USA) and a splitless injector with deactivated glass wool. Chromatographic conditions were as follows: 280 °C injector temperature, 1 μl pulsed splitless injection at oven temperature of 60 °C (1 min isotherm), then programmed at 30 K/min to 150 °C, at 6 K/min to 186 °C and finally at 4 K/min to 280 °C (30 min isotherm). Carrier gas velocity (Helium 5.0, Air Liquide, Böhlen, Germany) was 1.2 ml/min at constant flow. The mass spectrometer was operated in electron impact ionization mode (EI+, 70 eV) with a source temperature of 250 °C scanning from 30 to 500 amu (full-scan mode) or in single ion monitoring (SIM mode) recording typical masses from compounds fragmentation patterns. Five-point external calibration in the linear range from 0.25 ng/μl to 5 ng/μl was used to quantify target analytes. Each sample was spiked with benzo[a]pyrene-d_{12} as internal standard to correct results for errors due to differences in sample-volumes and injection. The instrumental limits of detection (LOD) defined as three times the signal-to-noise ratio were in the range from 0.7 pg/μl to 117 pg/μl and the limit of quantification (LOQ) defined as ten times the signal-to-noise ratio was in the range from 2.4 pg/μl to 390 pg/μl.

2.3. Extraction of fish tissue and extracts processing

All experiments regarding matrix effects were conducted using extracts of tissue from two different fish species. Frozen rainbow trout (*Onchorhynchus mykiss*) and salmon (*Salmo salar*) were bought at a local supermarket. The thawed fish muscle tissues were minced and freeze dried. 25 g of each dried tissue was ground with 50 g of diatomaceous earth (Isolute HM-N, IST Ltd., Hengoed, UK) using a porcelain mortar and pestle. The mixtures were filled in 100 ml ASE cells and extracted by means of an ASE 300 device (Dionex, Sunnyvale, CA). The extraction was performed with *n*-hexane:acetone 50:50 (v/v) at 80 °C and 10 MPa for three static cycles of 5 min (Table S2, Supplementary data). The joint extracts were concentrated using rotary evaporation to a volume of approximately 20 ml, transferred to a measuring cylinder, refilled to 25 ml and stored in the freezer at −20 °C until usage.

The extracts of fish tissue were saponified and derivatized to quantify the recoveries of fatty acid lipids during dialysis procedure. The extracts were mixed with 0.5 ml 1 M potassium hydroxide (p.a. grade, Merck Darmstadt, Germany) in methanol and incubated for 2 h at 60 °C in an oven. After cooling to room temperature 200 μl of 6 M hydrochloric acid (p.a. grade, Merck Darmstadt, Germany) were added and the fatty acids were extracted two times with 1 ml of *n*-hexane. A solution of 30 ng nonadecanoic acid (Fluka, Steinheim, Germany) and of hexan:chloroform:methanol (95:3:2, v:v:v) was added to the extract. The mixture was reduced to dryness using nitrogen, reconstituted in 1 ml of a solution of methanol:chloroform:38% hydrochloric acid (10:1:1, v:v:v) and incubated overnight at 60 °C in an oven. Finally, the fatty acid methyl esters were extracted three times using 0.5 ml of *n*-hexane:toluol (1:1, v:v). Aliquots of the raw fish extracts were treated with the same procedure to estimate the raw content of fatty acids lipids in triplicate.

2.4. Accelerated membrane-assisted cleanup

2.4.1. General description of the AMAC

Briefly, dialysis bags were tailor-made using commercially available cast polypropylene (CPP) (procast®, Zeisberger Süd-Folie GmbH, Asperg, Germany) and LDPE (Polymer-Synthese-Werk GmbH, Rheinberg, Germany) (half-)tubes with a membrane thickness of 50 μm and 80 μm, respectively. Pieces with a length of 10 cm were cut from stock roll, each membrane was cleaned for 24 h in a mixture of *n*-hexane:acetone (50:50) to remove excess oligomers, slip agents, plasticizers, stabilizers and other impurities, rinsed with fresh solvent and air dried for not more than 10 min [1]. Remaining compounds in blank samples that could be assigned to polymer fabrication were for example phthalates, 1,2-diphenylethane, 1,1′-[dithiobis(methylene)]dibenzene, 1-dodecanol, caprolactam, 1-(octyloxy)octane, n-butylbenzenesulfonamide, 1,1′-[dithiobis(methylene)]dibenzene, bis(2-ethylhexyl) adipate, and 13-docosenamide. These compounds were not disturbing instrumental analysis in this study.