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Focused ultrasound solid–liquid extraction for the determination of organic biomarkers in beachrocks

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ABSTRACT

Beachrocks are consolidated coastal sedimentary formations resulting mainly from the relative rapid cementation of beach sediments by different calcium carbonate polymorphs. Although previous works have already studied the elemental composition and the mineral phases composing these cements, few of them have focused their attention on the organic matter present therein. This work describes an extraction methodology based on focused ultrasound solid–liquid extraction (FUSLE), followed by analysis using large volume injection (LVI) in a programmable temperature vaporizer (PTV) combined with gas chromatography–mass spectrometry (GC–MS) in order to determine organics such as polycyclic aromatic hydrocarbons (PAHs) and biomarkers (hopanes), which can increase and confirm the information obtained so far. This goal has been achieved after the optimization of the main parameters affecting the extraction procedure, such as, extraction solvent, FUSLE variables (amplitude, extraction time and pulse time) and also variables affecting the LVI–PTV (vent time, injection speed and cryo-focusing temperature). The developed method rendered results comparable to traditional extraction methods in terms of accuracy (77–109%) and repeatability (RSD < 23%). Finally, the analyses performed over real beachrock samples from the Bay of Biscay (Northern Spain) revealed the presence of the 16 EPA priority PAHs, as well as some organic biomarkers which could increase the knowledge about such beachrock formation.

1. Introduction

Beachrocks are coastal sedimentary formations mainly resulting from the early in situ precipitation of $CaCO_3$ polymorphs between beach sediments [1–3]. The mechanisms proposed for beachrock formation include biological and physico-chemical processes. Recent literature has been dominated by those relating to CO_2 degassing owing to agitated marine waters and increasing temperature, mixing of alkaline fresh waters with sea water or chemical changes associated to decay of organic matter [4–8].

Previous works have generally focused their attention on the characterization of the mineral phases composing the cements of the beachrocks. As a consequence, several hypotheses about the implications of the formation of such deposits in the coastal zones have been described enabling the construction of different diagenetic models. To achieve this goal, scanning electron microscopy (SEM) [4], X-ray spectrometry and radiocarbon dating [9] have commonly been used, although in more recent studies Raman spectroscopy [2,3] has also been applied among other analytical techniques. Recently, the outstanding improvements of resolution in chromatographic techniques have opened new lines of research in beachrock characterization, being possible the analysis of the organic fraction preserved in the cementation. In fact, high resolution chromatographic techniques allow performing biomarker analyses using them as a tool to confirm and complement the information given by traditional techniques [10]. Aside from the chromatographic resolution, detection sensitivity and selectivity are other two essential parameters to be considered if the detection of biomarker compounds at trace levels (often present at sub ng g^{-1} quantities) is required. The use of large volume injection (LVI) in a programmable temperature vaporizer (PTV) (as injection system) or the use of high sensitivity mass spectrometers (as detection system) allows us to fulfill with such objective.

Biomarkers are strongly useful as they can provide information about the organic matter in the source rock (source), environmental conditions during its deposition and burial (diagenesis), the thermal maturity experienced by a rock or oil (catagenesis), the degree of biodegradation, as well as some aspects related to the source rock mineralogy (lithology) and age [11]. In this sense,

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polycyclic aromatic hydrocarbons (PAHs) are interesting compounds to be determined. Although PAHs can be originated from natural processes (i.e., biomass burning, volcanic eruptions and diagenesis [12]) or from anthropogenic ones (i.e., coal and wood burning, petrol and diesel oil combustion, and industrial processes [13]), they are always emitted as a mixture, and the relative molecular concentration ratios between them are considered to be characteristic of a given petrogenic or pyrogenic origin [14]. Other biomarkers such as C_{27}–C_{35} pentacyclic triterpanes (hopanes) can indicate the conditions during the formation of sedimentary rocks, as for example, salinity, presence or absence of oxygen, microbial activity and so on [11]. For instance, the presence of gammacerane ($C_{30}H_{52}$) indicates hypersaline environments, is a reliable geochemical indicator for water column stratification in marine deposits, and has been found to be present in nature in vast amounts as components of bacteria and other primitive organisms [15]. Thus, the presence of such compounds within the cements could also confirm the influence of biologically mediated processes in the first stages of cementation.

Notwithstanding the remarkable information that could be obtained from the organic residues preserved in geological samples, few authors have studied the presence of organic compounds within the beachrock cements. Although Olcott Marshall et al. [16] determined biomarkers in other matrices such as Precambrian glacial sediments by means of an accelerated microwave extraction obtaining very promising results, there is a lack of analytical methodologies developed to determine organic biomarkers in beachrock samples. Moreover, most of the works dealing with the analysis of such organic compounds in similar matrices like sediments, sands, soils and rocks often use very time consuming extraction techniques. In this framework, conventional Soxhlet extraction is the most referenced technique in this area, regardless of the long extraction periods (i.e., 8–16 h), large amount of sample (i.e., 100–150 g) and large extractant volumes (i.e., 150 mL) required [17–20]. Other extraction methods such as microwave assisted extraction (MAE) [21] have shown to be adequate extraction techniques for several solid matrices allowing the simultaneous extraction of multiple samples, but it is still time consuming and high-priced. Accelerated solvent extraction (ASE) has also been used in similar matrices [22] but this technique is more expensive. In this respect, ultrasound based solid–liquid pretreatments drive effective extraction processes in shorter time, with less analyte looses during the sample pre-treatment and using a, safe, low-cost and eco-friendly methodology [23]. Currently, among the different ultrasonic processors, ultrasound bath (USB) is one of the most common instruments to accelerate the extraction of both organic and inorganic compounds from several matrices [24,25]. However, the poor sonication power of ultrasound baths has made the focused ultrasound solid–liquid extraction (FUSLE) systems to be increasingly popular since they provide 100 times higher sonication power, as well as higher reproducibility and efficiency. In this latter ultrasonic performance, the ultrasound energy is focused in the tip of a probe, typically made of titanium and immersed directly in the solution of the sample to be sonicated. Hence, the use of focused ultrasound energy provides a short, reproducible and quantitative extraction from different matrices [26–28], including organic compounds as target analytes [26]. Nevertheless, the use of FUSLE for the extraction of organic compounds from beachrock samples has not still been reported in the literature.

The main objective of the research was to develop a fast, cost-effective, eco-friendly and sensitive analytical method based in focused ultrasound solid liquid extraction in combination with analysis by means of LVI–PTV–GC–MS. As far as we know, this methodology has never been applied in the analysis of organic compounds (PAHs and organic biomarkers) from beachrock samples. Guidelines that provide the framework for green analytical chemistry (GAC) are needed to support this affirmation. In this sense, the proposed method can help to fulfill 10 of the 12 principles of GAC proposed in literature (i.e., integrate analytical processes and operations, generate as little waste as possible and treat it properly, minimize use of energy, implement automation and miniaturization of methods, increase safety for operator, avoid derivatization, minimize sample number and size, use multi-analyte method, and replace toxic reagents [29]). Finally, the developed method was applied to beachrock samples with the purpose of finding PAHs and biomarkers to identify probable sources of organic matter and shed some light on the diagenetic process of the sedimentary outcrops.

2. Materials and methods

2.1. Studied material

The beachrock samples used in the optimization of the analytical procedure and its assessment belong to Tunelboka cove, which is located at 43°N latitude (Bay of Biscay, Northern Spain). Thus, the beachrock is placed at an unusual temperate latitude setting, in contrast to the most documented beachrocks at tropical and subtropical emplacements [1,2]. More exactly, the cove is located in the surroundings of the Nerbioi-Ibaizabal estuary, an area marked by a strong industrialization background. This context induces a considerable heterogeneous character to the beachrocks, being the granulometric separation a helpful tool to somehow differentiate the cements between the framework grains [3]. Indeed, this finest particle size related to the cements is the target one in order to determine biomarkers, which will help to conclude whether microorganisms where involved in the formation of the beachrock outcrops and to understand the specific formation conditions at this latitude.

2.2. Reagents and materials

Laboratory material was carefully cleaned with abundant pure water (<0.2 $\mu S\ cm^{-1}$, Millipore, USA) and without using detergent to avoid possible contamination produced by detergent residues. The material was sonicated under clean acetone (Q.P., Panreac Química, Spain) for an hour and then rinsed with ultrapure water (<0.057 $\mu S\ cm^{-1}$, Milli-Q model, Millipore, USA). Finally, the glass material was dried in an oven at 400 °C for 4 h.

Polycyclic aromatic hydrocarbon mix containing Naphthalene (Naph), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flr), Pyrene (Pyr), Benzo(a)anthracene (BaA), Crysene (Cry), Benzo[b]Fluoranthene (B[b]F), Benzo[k]Fluoranthene (B[k]F), Benzo[a]Pyrene (B[a]P), Indeno[1,2,3-cd]pyrene (IcdP), Dibenzo[a,h]anthracene (DahA) and Benzo[g,h,i]perylene (BghiP) at 2000 $\mu g\ mL^{-1}$ was purchased from Supelco (Sigma–Aldrich, Germany). A mix of five deuterated PAHs ([$^2H_2$] Naphthalene, [$^2H_{10}$] Acenaphthene, [$^2H_{10}$] Phenanthrene, [$^2H_{12}$] Chrysene and [$^2H_{12}$] Perylene) supplied by Supelco (Sigma–Aldrich, Germany) was used as surrogate. Mixed fresh solutions with ≈50 $\mu g\ g^{-1}$ of each target compound were monthly prepared and stored in amber vials at −20 °C. Dilutions of these stock solutions were daily prepared in *n*-hexane according to the experimentation.

The used solvents, *n*-hexane (Hex), dichloromethane (CH_2Cl_2) and methanol (MeOH) (all HPLC grade, 99.8%) were purchased from LabScan (Dublin, Ireland). Copper (powder Cu), used in order to eliminate the high amounts of sulfur present in samples, was acquired from Merck (Darmstadt, Germany).