Green Extraction Techniques Applications in Different Fields

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Contributor: Jorge Pereira , José Sousa Câmara , Rosa Perestrelo , Cristina Berenguer , , Abuzar Kabir , Cristina M.R. Rocha , José Couto Teixeira

Green extraction techniques (GreETs) emerged in the last decade as greener and sustainable alternatives to classical sample preparation procedures aiming to improve the selectivity and sensitivity of analytical methods, simultaneously reducing the deleterious side effects of classical extraction techniques (CETs) for both the operator and the environment. The implementation of improved processes that overcome the main constraints of classical methods in terms of efficiency and ability to minimize or eliminate the use and generation of harmful substances will promote more efficient use of energy and resources in close association with the principles supporting the concept of green chemistry.

green extraction techniques

microextraction techniques

sample preparation

1. Biological Samples

The application of GreETs to the clinical field has increased consistently since the beginning of the century [1]. This mostly includes body fluid samples containing lower-molecular-mass organic molecules, less than 500 g/mol, comprising drug analytes, metabolites, environmental exposure contaminants, poisons, tissues, and endogenous substances [1]. These biological samples present great complexity and moderate-to-high levels of protein, thus requiring robust sample preparation approaches able to simplify and isolate the target analytes from the matrix $^{[2]}$. As discussed in more detail in the previous sections, traditional sample preparation methods are not particularly tailored for clinical applications because they are time-consuming and require various steps and extensive clean-up before analysis. In contrast, most GreETs require low sample amounts, very low or no solvent at all, and simple, fast, and user-friendly systems that can be easily automated [2]. These advantages made SPME, μ SPE. MEPS. MSPE, just to name a few GreETs, particularly suitable to process biological samples. Moreover, they also allow spanning a wide range of analytes with different properties, such as drugs for clinical and forensic toxicology assays, pharmacokinetic studies, biochemical analysis, pharmaceuticals, in vivo applications, and metabolomics ^[2]. SPME and its different formats are particularly efficient in this field of application because they often require minimum sample pretreatment and can be easily coupled to analytical instruments (e.g., CG and LC), providing an enhanced extraction capacity and simultaneous quantification of different compounds with overall sensitivity. This includes the simultaneous identification of drugs of abuse (e.g., amphetamines, barbiturates, methadone), psychoactive substances, pharmaceuticals (e.g., antidepressants, antiepileptic agents, steroids, anorectic agents, corticosteroids, anaesthetics), substances that affect the adrenergic system, nonsteroidal anti-inflammatory substances, and so forth ^[3]. Among the different biological matrices, microextraction of urine samples has the advantage of minimum processing, often not requiring any centrifugation or filtration before extraction. This minimizes sample handling and improves method precision. Additionally, it is suitable for a wide range of sample volumes, including volumes as small as 50 µL, and even for sampling when the volume is not accurately known. Diverse types of GreETs using urine are available in the literature, SPME, µSPE, and MEPS being the most often reported ^[2]. The use of GreETs with blood sampling is also advantageous, particularly when this allows the elimination of blood-withdrawal steps from the analytical workflow, as with SPME. GreET usage also reduces the risk of analyte degradation and matrix changes due to enzymatic conversion, as well as fast sample collection and clean-up. Different examples of applications involving blood sampling using GreETs can be found in the literature, such as VOCs (SPME $[\underline{4}]$), polycyclic aromatic hydrocarbons (PAHs, pipette-tip SPE $[\underline{5}]$), Ni and Pb (μ SPE $[\underline{6}]$), opiates (MEPS \square), and antidepressants (FPSE \square). SPME has also been reported in in vivo assays with biological matrices like tissues. This can be performed with a removed tissue portion (ex vivo), direct in vivo measurement, exposing the BioSPME needle to the tissue or even inserting the probes directly into the tissue. Regarding this, Musteata ^[9] observed that microdialysis and SPME were not only appropriate for tissue sampling but also complementary to each other for in vivo sampling and ex situ analysis. By using this approach, the probe extracts only a slight fraction of the free analyte, minimizing disturbances of chemical equilibrium and allowing multiple measurements of analyte concentrations under physiological conditions. Moreover, the accurate determination of analyte concentration is unaffected by the sample volume. Finally, the technique is open to miniaturization, allowing its application within small living systems, sample storage and transportation, and easy coupling to portable instrumentation ^[2]. An example of such an approach was reported by Cudjoe, et al. ^[10], which used SPME to monitor neurotransmitter changes in the striatum of a rat brain after dosing antidepressants, variations in serotonin concentrations due to deep-brain stimulations, and distribution of pharmaceuticals in the striatal region and cortex. This elegant experiment shows that SPME can also be very useful in metabolomics assays, particularly at the initial stage of biomarker discovery in medical diagnosis. It is also very relevant to the quantification of different compounds simultaneously, which enables the simultaneous monitoring of drugs in complex treatments. This is possible because GreETs coupling with chromatographic methods, can be easily achieved, allowing the analysis of a whole pharmacopoeia of drugs, such as anticancer, antibiotic, antidepressant, analgesic, anti-inflammatory, steroid, and neurotransmitter drugs. This can help to provide earlier detection of the disease, which is imperative for a successful clinical treatment, especially in some oncologic diseases, where an early diagnosis is crucial for the survival of the patient without suffering severe impacts on health and life quality. FPSE is a very promising GreET having a key advantage regarding other microextraction approaches, allowing a direct analyte extraction with no sample modification ^[11]. Since its introduction in 2014, many examples of applications involving biological samples have been reported in the literature, such as the cow and human breast milk sample clean-up for screening bisphenol A and residual dental restorative material ^[12]; the simultaneous monitoring of inflammatory bowel disease treatment drugs ^[13] and anticancer drugs ^[14] in whole blood, plasma, and urine; or the assessment of radiation exposure [15]. The use of magnetic nanoparticles as microextraction sorbents in MSPE also results in a very simple and efficient extraction procedure because the sorbent can be tailored to extract specific analytes, and the sorbent-retained analyte complex can be easily recovered from the solution using a magnetic field or magnet ^[16]. MSPE has been used to extract different drugs from urine, such as nonsteroidal anti-inflammatory drugs

(NSAIDs) ^[17], methadone ^[18], pseudoephedrine ^[19], fluoxetine ^[20], and statins ^[21], as well as antiepileptic drugs ^[22] or ibuprofen ^[23] from plasma. GreETs involving liquid-phase sorbents, such as DLLME, are also often reported in the literature. This format, mostly assisted by ultrasounds (UA-DLLME), allows the usage of a myriad of extraction solvents, and consequently, the repertoire of applications is very broad. Mabrouk et al. ^[24], for instance, used UA-DLLME to extract three gliflozins (antidiabetic drugs) from plasma.

2. Food Samples

Food analysis is of great importance since ingestion of a growing number of compounds intentionally or not added to food can represent a risk to our health. However, beyond food safety, consumers are also more aware of the nutritional value of food and are also interested in its composition, particularly regarding the presence of bioactive compounds. For these reasons, efficient methodologies for the identification and quantification of all these analytes are required. Accordingly, GreETs have been used in the sample preparation procedures of different food matrices to extract and preconcentrate target analytes to a sufficient level to allow their analysis $\frac{25}{25}$. The µSPE technique, for instance, has been used in the determination of aflatoxins $\frac{[25]}{2}$, pesticides $\frac{[26]}{2}$, trace metals $\frac{[27]}{2}$, and pollutants, such as bisphenol A ^[28] and PAHs ^[29], in a variety of food products. Additionally, it aided in the identification and guantification of rosmarinic acid in medicinal plants ^[30] and vitamin D3 in bovine milk ^[31]. MEPS is another GreET that has been employed in the analysis of foodstuffs, including the identification of herbicides in rice [32], insecticides in drinking water ^[33], pesticides in apple juice and coffee ^[34], antibiotics ^[35] and steroids ^[36] in milk, parabens in vegetable oil [37], PAHs in apple [38], caffeine in drinks [39], and polyphenols in baby food [40]. SPME has been widely used to study the volatile composition of several foods, including walnut oils [41], hongeo [42], melon ^[43], and dairy products ^[44]. Moreover, this technique has also been used to determine the composition of specific analytes, such as the x-ray induced markers 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in irradiated dairy products [45], the contaminants 1,4-dioxane and 1,2,3-trichloropropane [46], acrylamide [47], organophosphorus pesticides ^[48], phthalates ^[49], synthetic phenolic antioxidants ^[50], and xanthines ^[51]. MSPD has been reported in the literature for the extraction of flavonoids [52], polyphenols [53], mangiferin, and hyperoside in mango-processing waste [54], ergosterol in edible fungi [55], and pharmacologically active substances in microalgae ^[56]. This methodology has also been applied for pesticide ^[57] and sulfonylurea herbicide ^[58] extraction in several food matrices. MSPE allowed the extraction of trace metals in food products ^[59]. Moreover, studies have shown that this technique can be used for the determination of acrylamide $\frac{5}{6}$, bisphenols $\frac{8}{2}$, PAHs $\frac{60}{2}$, plant growth regulators [61], and caffeine [62]. FPSE is another GreET that has been shown to be very useful for the determination of several classes of pesticides in foods [25]. Other analytes studied using this technique include bisphenol A ^[63], oligomers ^[64], PAHs ^[65], steroid hormone residues ^[66], and tetracycline residues ^[67]. DLLME has been vastly applied for the determination of trace metals [68], pesticides [25], chloramphenicol [69], and nonsteroidal anti-inflammatory drugs ^[70] in different foods. µQuEChERS was employed in the extraction of several analytes from foods, ranging from pesticide residues in wine $\frac{71}{2}$ and PAHs in coffee and tea $\frac{72}{2}$ to polyphenols in baby food [73] and pyrrolizidine alkaloids in oregano [74]. The application of SDME was proved to allow the determination of unfavorable compounds and elements in foods, such as drug metabolites ^[75], acrylamide ^[76], ammonia ^[77], ethyl carbamate ^[78], formaldehyde ^[79], tartrazine ^[80], and Cu(II) ^[81]. Similarly to SDME, SFOME can be used for the

detection of trace metals ^[82], as well as of β-lactam antibiotic residues ^[83] and organochlorine pesticides ^[84]. PEAE has been applied for the extraction of different bioactive compounds ^[85], including phenolic compounds ^[86], carotenoids ^[87], procyanidins ^[88], and sulforaphane ^[89]. The use of SFE has been used for the extraction of several antioxidant and antibacterial compounds from feijoa leaf ^[90], fatty acids and oils from Indian almonds ^[91], oleoresins from industrial food waste ^[92], and polar lipid fraction from blackberry and passion fruits ^[93]. Additionally, SFE was employed for the extraction of several classes of bioactive compounds, including anthocyanins ^[95], fatty acids ^[96], hesperidin and narirutin ^[97], phenolic compounds ^[98], and scopoletin, alizarin, and rutin ^[99]. The extraction of antioxidant protein hydrolysates from shellfish waste ^[100] and pectic polysaccharides from apple pomace has been also previously accomplished by SWE ^[101].

3. Environmental Samples

Most environmental samples have complex matrix compositions and involve the determination of trace and ultratrace analytes [102]. For instance, the determination of PAHs in water samples or pesticide analysis is challenging due to their very low concentrations [102][103]. This requires efficient clean-up and enrichment procedures before the analytes' analysis [103]. MEPS seems to be tailored for these requirements and has been applied in the analysis of benzene, phenol and their derivates $\frac{[104]}{104}$, diazinon $\frac{[105]}{105}$, La³⁺ and Tb³⁺ $\frac{[106]}{106}$, organophosphorus pesticides $\frac{[107]}{107}$ in water samples, fipronil and fluazuron residues in wastewater [108], and PAHs in the most diverse samples, including Antarctic snow ^[109], and in the detection of phthalates in tap and river water ^[110]. SPME is eventually one of the most used sample extraction procedures and has been applied for the detection of different pesticides in water ^[111], microplastic in coral reef invertebrates ^[112], PAHs in rainwater ^[113], and volatile organic compounds (VOCs) in wastewater [114]. Molecularly imprinted polymers (MIPs) have also been employed in the extraction of polychlorinated aromatic compounds from environmental samples. Some applications include the use of MIPs in the analysis of 2-chlorophenol [115], 2,4-dichlorophenoxyacetic acid [116], and endosulfans [117] in water samples and in the determination of organochlorine pesticides in environmental samples [118]. This methodology has also been reported in the preparation of soil samples to increase the extraction efficiency of triazine herbicides [119]. Multisphere adsorptive microextraction (MSAµ) has been applied in the extraction of caffeine, acetaminophen ^[120], pharmaceuticals, sexual steroid hormones, and antibiotics [121] in water samples. QuEChERS is known as the Swiss knife of extraction. Its µQuEChERS version is even more greener and includes applications such as the detection of insecticides in guttation fluids [122], pesticides in arthropods and gastropods [123], and VOCs in zebrafish [124].

LPME techniques, such as SDME and SLLME, use small volumes of organic solvents to extract the analytes ^[125]. SDME has gained a lot of interest in the last few years and is mostly used for the determination of trace analysis in environmental matrices, including Cu(II) in tap and seawater ^[81], PAHs in tap water ^[126], ranitidine in wastewater ^[127], and V(V) in water samples ^[128]. DLLME is another efficient microextraction procedure, and its ultrasound-assisted (UA) DLLME variation has been adopted in several environmental matrices for the analysis of aromatic amines ^[129], Cd ^[130], Cr ^[131], dyes ^[132], herbicides ^[133], polybrominated biphenyls ^[134], pyrethroid insecticides ^[135],

and tetracycline ^[136] in water samples. SFE was applied to environmental matrices for the analysis of Ag in electronic waste ^[137], petroleum biomarkers in tar balls and crude oils ^[138], petroleum hydrocarbons in soil ^[139], and solanesol in tobacco residues ^[140]. In turn, SWE has been successfully used for the extraction of Co, Li, and Mn in spent lithium-ion batteries ^[141], crude oil in soil ^[142], oil shale in mines ^[143], and VOCs in sewage sludge ^[144].

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