ULTRASOUND-ASSISTED EMULSIFICATION MICROEXTRACTION IN THE ENVIRONMENTAL AND FOOD ANALYSIS

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Abstract: Ultrasound-assisted emulsification microextraction (USAEME) has gained widespread recognition as a simple, fast, and miniaturized sample preconcentration technique. In USAEME microquantities of solvents are consumed, and therefore it is becoming a cheap and environmentally friendly technique for separating. Moreover, the advantages of this technique are simplicity of operation, low cost, high recovery and enrichment factors. USAEME is proposed as an alternative to other extraction techniques such as SPE, SPME and LPME for the analysis of environmental samples including bottled, tap, river, municipal swimming pool, sewage and seaport water samples. It has been applied to determine a variety of inorganic and organic compounds in different matrices. This review summarizes the USAEME principles, various parameters of the technique, recent trends and selected applications.

1. Introduction

Analysis of environmental samples is an important issue due to a variety of analytes and a variability range of concentrations, as well as the complicated composition of the matrix. Components of a sample may be a source of interference. Their presence will thus hinder further analytical procedure. Accordingly, it is appropriate to carry out the isolation and enrichment step in the preparation of the analytes of the sample for further analysis.

Liquid-liquid extraction (LLE) is the oldest preconcentration and isolation technique in analytical chemistry. However, it is time-consuming and requires using large amounts of organic solvents. Modern analytical chemistry offers a large selection of extractive techniques. Extraction is often applied to the liquid phase together with various modifications (e.g. Single drop microextraction – SDME, Hollow fibre liquid phase microextraction – HF-LPME, Micellar extraction ME, Supercritical fluid extraction – SFE, Ultrasound-assisted emulsification microextraction – USAEME;
The process of isolating analytes from a sample is often supported by additional factors such as increased pressure, temperature, and microwave radiation. Modifications to the classical liquid-liquid extraction also allow a volume decrease of the analyzed sample, reducing the driving time analysis and partial or total automation of the extraction process. New technical solutions combine both miniaturization of the apparatus and reducing the number and amount of chemical agents, especially toxic organic solvents.

The purpose of this study is to describe one of the mentioned preconcentration techniques – USAEME. The main focus is on the type of extraction solvent and the application for analysis.

2. USAEME principle

The USAEME technique is based on the introduction of a small amount of organic solvent to the water sample (Fig. 1). The assembly is subjected to ultrasound until the organic solvent disperses into small droplets. The use of ultrasound emulsification facilitates and accelerates the process of mass transfer between two immiscible phases. This leads to an increase in extraction efficiency in the shortest possible time. Next, the sample is centrifuged. The separated organic layer is ready for the determination of appropriate analytical methods (Fontana et al., 2009).

The mechanism used in the emulsification USAEME effect is based on cavitation. Bubbles are formed as a result of intense shock wave and a high-speed stream. Such microstream of bubbles may cause disruption of the solvent droplet, thereby improving the emulsification phenomenon by generating a smaller size of the dispersed phase droplets. This leads to a substantial increase in the transfer process of the compounds and better surface contact between the two immiscible liquids.

It is worth mentioning that microquantity solvents are consumed in the USAEME technique, and therefore it becomes a cheap and environmentally friendly technique for separating. Moreover, the advantages of the USAEME technique are: simplicity of operation, low cost, high recovery and enrichment factors.

In the USAEME technique emulsification under ultrasonic radiation is necessary. Therefore, selection of a suitable extraction solvent is limited by several characteristics. Mainly, the extraction solvent has to fulfill several requirements such as: extraction ability, lower density than water and low water solubility. Many of the solvents are less dense than water, thus they are not used in emulsification based microextraction techniques. In general, the most popular extraction solvents are: chloroform, carbon tetrachloride, toluene, chlorobenzene, and undecanol (Andruch et al., 2013).

In all cases, the effect of the extraction solvent volume is examined. Mainly, 100 µl of the selected solvent are used. The influence of the volume ratio between the two liquid phases (water and organic solvent) is also considered. Using a smaller volume of organic solvent in comparison to the water sample lets us reach a higher enrichment factor (EF). EF is defined as the ratio between the analyte concentration after the separation process (C₁) and the initial concentration of the analyte (C₀) within the sample: EF = C₁/C₀. EF value ranged from several dozen to several hundred, which mainly depended on the kind of analytes and volume of the sample.

Various parameters that affect the extraction efficiency, such as salt addition and ionic strength, ultrasound emulsification time and extraction temperature are also
investigated in the USAEME technique.

In general, addition of salt to the sample can decrease the solubility of analytes in the aqueous phase and promote the transfer of the analytes towards the organic phase. Mostly, sodium chloride is selected and 0 – 5% of an electrolyte concentration is used. The concentration of an electrolyte in the aqueous solution is one of the factors which is evaluated. The results often revealed that the extraction efficiency increased with the concentration of NaCl to 1%. Nevertheless, Wu et al. (2010) showed that the addition of salt had no significant effect on the extraction recoveries for all the target analytes. Maybe, it is because a non-ionic surfactant was used.

Proper sonication time is needed to guarantee equilibrium extraction and mass transfer from the aqueous solution to the organic phase. The results from publications on the subject indicated that the extraction equilibrium can be mostly attained within 10 min. The other half of the argument states that prolonging sonication time could result in descent of extraction efficiency, especially when using ionic liquids. It is because of volatilization of the analytes and dissolution of the IL in the aqueous solution because of the abundant sonication heat generated (Liang et al., 2013). Cabaleiro et al. (2011) show the influence of no shaking (only centrifugation), magnetic shaking, bath sonication and cup-horn sonication for achieving complete emulsification. The comparison of the suitability of ultrasound energy in these microextraction procedures suggests that longer times (up to 16 min) are needed when magnetic or bath sonication is used. When 6 min was fixed as shaking time for all systems, higher extraction efficiency was achieved in the sonoreactor. It can be concluded that sonication plays an important role in the USAEME process and it accelerates the transfer of the analyte to the microquantity of the organic solvent.

Temperature can also affect the mass transfer rate and the contact area between the sample solution and the extraction solvent. This effect is usually studied in the

![Fig. 1. A schematic diagram of the USAEME process: (a) sample solution with analytes, (b) addition of extraction solvent into the water sample, (c) ultrasound-assisted emulsification, (d) phase separation after centrifugation, and (e) analysis of a small volume of organic phase containing the analyte.](image-url)
range of 20 – 60 °C. In some cases, emulsification is easily achieved and sustained during the whole extraction time. For the convenience of the experiments, extractions were carried out at room temperature (25 ± 2 °C).

3. USAEME development and application

The first application of USAEME was performed for extraction and preconcentration of synthetic musk fragrances, phthalate esters and lindane in water samples and their determination by gas chromatography coupled to mass spectrometry (GC/MS) (Regueiro J., 2008). Compounds were extracted during 10 min in an acoustically emulsified media formed by 100 µl chloroform and 10 ml sample (enrichment factor equals 100). The obtained results indicated that limits of detection were at the pgml⁻¹ level for most compounds, and at the ngml⁻¹ level for most ubiquitous phthalate esters.

USAEME has been widely used for the isolation of organic compounds and metal ions in environmental and food samples (Fontana and Altamirano, 2010; Ge and Lee, 2012; Najafi et al., 2012; Vinas et al., 2010). Table 1 presents selected examples of application of this preconcentration technique. The results show the use of USAEME for extracting of a wide range of organic and inorganic compounds from water and food samples.

Firstly, in a conventional USAEME process typical solvents were used. In the novel approach ionic liquids (IL) are used as extractant solvents instead of the organic ones. IL-USAEME combination with high performance liquid chromatography has been developed for the determination of four fungicides (azoxystrobin, diethofencarb, pyrimethanil and kresoxim-methyl) in water samples (Liang et al., 2013). Three imidazolium-ILs with PF₆⁻ as anion, including [C₄MIM][PF₆], [C₆MIM][PF₆] and [C₈MIM][PF₆] were investigated wherein 40 µl of [C₈MIM][PF₆] was chosen as the most efficient for this purpose. In another example, 60 µl of an ultra-hydrophobic 1-hexyl-3-methylimidazolium tris (pentfluoroethyl) trifluorophosphate [HMim][FAP] was used as an extractant to determine organic ultraviolet filters in water samples by the HPLC-UV method (Ge and Lee, 2012).

There is a possibility that besides ionic liquids surfactants can be used. The procedures based on the use of micellar-ionic liquids (ILs) as an extractant are proposed for triclosan determination in troublesome matrices such as cosmetics and wastewaters (Cabaleiro et al., 2011). An improvement in the extraction of the derivative was achieved when sodium dodecylsulphate (SDS) was mixed with ionic liquids ([C₄MIM][PF₆] and [C₈MIM][PF₆]). Surfactant aggregates or micelles can form in different types of solvents, including ILs. When micellar solutions formed upon addition of 100 m M anionic surfactant SDS was tried as an extractant phase, efficient extraction of the derivative was observed.

Non-ionic surface active compounds can also be used. Tween 20 was adapted as an emulsifier, and chlorobenzene and chloroform were used as a dual extraction solvent for determination of six carbamate pesticides (metolcarb, carbofuran, carbaryl, pirimicarb, isoprocarb and diethofencarb) in river, reservoir and well water samples. Under optimum conditions, enrichment factors were in the range between 170 and 246 (Wu et al., 2010).
Tab. 1. Selected examples of using USAEME to extract different analytes from food and water samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>Limit of detection (LOD)</th>
<th>Recovery [%]</th>
<th>Determination method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>organolead and organomanaganese compounds</td>
<td>seawater</td>
<td>7.41 ng l⁻¹</td>
<td>84-118</td>
<td>GC (MS)</td>
<td>(Campillo et al., 2014)</td>
</tr>
<tr>
<td>phenolic compounds</td>
<td>olive oils</td>
<td>0.004-0.47 mg kg⁻¹</td>
<td>91-115</td>
<td>HPLC (DAD)</td>
<td>(Reboredo-Rodríguez et al., 2014)</td>
</tr>
<tr>
<td>polycyclic aromatic hydrocarbons</td>
<td>fish oil</td>
<td>0.02-2.6 µg kg⁻¹</td>
<td>70-108</td>
<td>HPLC (FLD)</td>
<td>(Yebra-Pimentel et al., 2013)</td>
</tr>
<tr>
<td>picric acid</td>
<td>water</td>
<td>7 µg l⁻¹</td>
<td>-</td>
<td>UV (Vis)</td>
<td>(Andruch et al., 2013)</td>
</tr>
<tr>
<td>carbamate-acetate</td>
<td>alcoholic-beverages</td>
<td>0.03 µg l⁻¹</td>
<td>80.5-87.9</td>
<td>GC (MS/MS)</td>
<td>(Liao et al., 2013)</td>
</tr>
<tr>
<td>bisphenol A</td>
<td>drink</td>
<td>38 ng l⁻¹</td>
<td>≥ 82</td>
<td>GC (MS)</td>
<td>(Andruch et al., 2013)</td>
</tr>
<tr>
<td>organochlorine pesticides</td>
<td>water</td>
<td>0.8-10 ng l⁻¹</td>
<td>77.7-120.3</td>
<td>GC (MS)</td>
<td>(Zhang and Lee, 2012)</td>
</tr>
<tr>
<td>selene</td>
<td>water</td>
<td>0.11 ng ml⁻¹</td>
<td>-</td>
<td>GC (FID)</td>
<td>(Najafi et al., 2012)</td>
</tr>
<tr>
<td>heavy metals</td>
<td>water</td>
<td>0.13-0.52 µg l⁻¹</td>
<td>90.3-105.5</td>
<td>ICP (OES)</td>
<td>(Sereshti et al., 2012)</td>
</tr>
<tr>
<td>phenol derivatives</td>
<td>wine</td>
<td>0.032-0.064 µg l⁻¹</td>
<td>86</td>
<td>GC (MS)</td>
<td>(Pizarro et al., 2011)</td>
</tr>
<tr>
<td>diethofencarb and pyrimethanil fungicides</td>
<td>water, fruit juices</td>
<td>0.01 µg l⁻¹</td>
<td>86-117</td>
<td>HPLC-DAD/MS</td>
<td>(Cheng et al., 2011)</td>
</tr>
<tr>
<td>sulfur compounds</td>
<td>wine</td>
<td>0.36-1.67 ng ml⁻¹</td>
<td>91.99-125.87</td>
<td>GC (MS)</td>
<td>(Jofré et al., 2010)</td>
</tr>
<tr>
<td>polycyclic aromatic hydrocarbons</td>
<td>water</td>
<td>0.001-0.036 µg l⁻¹</td>
<td>92</td>
<td>GC (MS)</td>
<td>(Ozcan et al., 2010)</td>
</tr>
<tr>
<td>2,4,6-trichloroanisole</td>
<td>water</td>
<td>1 ng l⁻¹</td>
<td>≥ 94</td>
<td>GC (MS/MS)</td>
<td>(Fontana and Alamirano, 2010)</td>
</tr>
<tr>
<td>strobulin and oxazole</td>
<td>juice, fruit</td>
<td>0.006-0.01 ng ml⁻¹</td>
<td>80-119</td>
<td>GC (MS)</td>
<td>(Vinues et al., 2010)</td>
</tr>
<tr>
<td>nickel (II)</td>
<td>water</td>
<td>0.34 ng ml⁻¹</td>
<td>97-103</td>
<td>FAAS</td>
<td>(Mohammadi et al., 2010)</td>
</tr>
<tr>
<td>polybrominated diphenyl ethers</td>
<td>water</td>
<td>1.2 pg ml⁻¹</td>
<td>≥ 96</td>
<td>GC (MS)</td>
<td>(Fontana et al., 2009)</td>
</tr>
<tr>
<td>cadmium</td>
<td>water</td>
<td>0.91 µg l⁻¹</td>
<td>96.5-101.7</td>
<td>FAAS</td>
<td>(Ma et al., 2009)</td>
</tr>
</tbody>
</table>
4. Conclusion

In the present study, the ultrasound-assisted emulsification microextraction technique was described as a useful tool for preconcentration and isolation steps in analytical procedures. USAEME provides high recovery and enrichment factor within a short time. In comparison to other techniques such as: SPME, SPE and LLE, USAEME is fast, simple and inexpensive. Using USAEME as a preconcentration step can provide good repeatability, high enrichment factors and good recoveries with a short analysis time.

Most applications of USAEME were performed for the analysis of environmental water samples in the first years of method development. However, a number of publications are now available also in food science.

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