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Deep eutectic solvents incorporated in a polymeric film for organophosphorus pesticide microextraction from water samples

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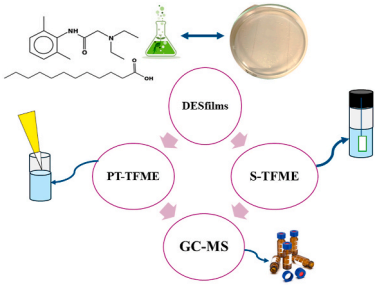
HIGHLIGHTS

- A film with a deep eutectic solvent in cellulose triacetate matrix has been prepared.
- Suspended thin film microextraction method with DES-based film evaluated.
- Organophosphorus pesticides determined in different water matrices.
- Pipette tip set-up useful for pesticide monitoring.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Organophosphorus pesticides (OPPs) were extensively used in agriculture. Due to their adverse effect, there is a need for sensitive and reliable methods to determine these agrochemicals. Microextraction techniques (ME) afford the opportunity to substantially reduce the amount of organic solvent used in classical extraction methods for pesticide analysis. Moreover, deep eutectic solvents (DES) made of components of natural origin, have been applied in microextraction techniques as a green alternative to organic solvents. The combination of thin film microextraction and DES can be seen as an alternative for thin film microextraction of OPPs from water samples.

Results: We describe a thin film microextraction-GC-MS method for the determination of OPPs from water samples. The thin film was prepared by solvent casting using cellulose triacetate (CTA) as the polymer and a deep eutectic solvent as the extracting phase. Lidocaine, menthol, dodecanoic acid, and camphor were tested as the components for DES-based film. With a film containing 70 % (w) of CTA and 30 % of the DES dodecanoic acid: lidocaine, quantitative results for the extraction of an OPPs mix were achieved. Then, the elution was performed with 2 mL of ethyl acetate. The validation of the TFME method was performed with a piece of the film suspended in 20 mL of sample solution with a contact time of 1 h. Limits of detection in the low μg L^{-1} range were obtained using a single quadrupole mass analyser. The thin film with pipette tip configuration was tested and preliminary results for chlorpyrifos were satisfactory.

Significance: This represents the first approach to use polymeric films made of CTA and DES for TFME of OPPs, in two configuration the suspended film and pipette tip.

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1. Introduction

Organophosphorus pesticides (OPPs), acting as cholinesterase inhibitors, are extensively used in agriculture all over the world. OPPs gained popularity as a substitute as they were cheap and readily available, had a wide range of efficacy, could combat a large number of pest species, and had a shorter environmental half-life than their organochlorine predecessors [1]. Camacho-Pérez et al. [2] have reported that, in 2020, pesticides applied globally reached up to 3.5 million tons, of which approximately one-third consisted of organophosphorus pesticides. Nevertheless, in recent years most of the OPPs have been banned in different countries. According to European Union, through European Commission Regulations No 2020/18 and 2020/17, chlorpyrifos, a widely used OPP, was withdrawn from use [3].

Due to the significant number of new studies providing evidence of the adverse effect of pesticides, there is a need for more sensitive and reliable methods that are suitable to determine these agrochemicals in a multi-residue approach. Several sample preparation methods use classical extraction techniques that are accepted by the Environmental Protection Agency (EPA) for pesticides. However, these methods were developed a long time ago and they typically do not reach the requirements for current analytical methods to be considered as green [4].

Microextraction techniques, which are characterized by a small amount of extraction phase compared to the volume of the sample, afford the opportunity to substantially reduce the amount of organic solvent used while still achieving similar or better results than traditional extraction techniques such as solid phase extraction (SPE) and liquid–liquid extraction (LLE). The volume of the extraction phase, which is inconsequential in relation to the overall volume of the sample, permits rapid non-exhaustive extraction, in some cases non-depletive, that can easily be quantitated using a variety of calibration methods. Moreover, analytes from the sample matrix are extracted in their "free-form" (non-bound or free-concentration), giving the opportunity for the analysis of bio-available analytes in various matrices [5].

Different types of microextraction techniques (single drop, dispersive, hollow-fibre liquid phase microextraction) have been developed for the determination of organic pollutants.

In the early 2000s thin film microextraction (TFME) was developed as an alternative to solid phase microextraction (SPME) [6]. In this configuration, a film is used that is immersed in the aqueous solution for the microextraction of the target analytes. One of the most significant characteristics of TFME is the geometry that enhances the sensitivity of analysis using a larger volume of extractive phase compared to the SPME counterpart [4]. With regard to the film, different materials have been investigated. Initial works mainly used polydimethylsiloxane (PDMS) as the extracting material. However, due to the lower affinity of this material for less lipophilic compounds, new materials and new preparation techniques have been explored [6]. In our research group, polymeric membranes containing plasticizers or modified with multiwalled carbon nanotubes (MWCNTs) have been successfully prepared and applied for TFME of organic pollutants in water samples [7–9]. The characteristics of polymeric membrane-based TFME allow it to play a crucial role as an environmentally friendly analytical alternative that also has the advantages of being relatively low cost and of being easy to manipulate.

Disposable pipette tip extraction (DPX) was initially developed as a solid-phase extraction (μ-SPE)-based device in which a small amount of sorbent is placed inside a pipette tip between two barriers: one in the narrow bottom and the other near the top of the tip [10]. This miniaturized format results in small solvent elution volumes compared to conventional SPE and high throughput parallel sample processing. The extraction of compounds is not dependent of the sample flow-rate using this configuration and on-site application is facilitated [10,11]. Usual sorbents for DPX are solid sorbents such as C18, which is used for the purification of biomolecules, conducting polymers (polyaniline), nanomaterials deposited over paper, and nanocomposites [10–13]. Environmental applications are less common, and the possibility of using TFME together with DPX has yet to be explored.

In recent years, there has been an increasing focus on replacing conventional extraction techniques with so-called "green" extraction techniques. This started with the introduction of green analytical chemistry (GAC), given a framework with the 12 principles formulated by P. Anastas in 1998, whose main concern is to create environmentally friendly analytical techniques, and especially extraction techniques. Deep eutectic solvents (DES) fit perfectly in the Green Analytical Chemistry (GAC) principles [14]. DES consist of two or more components that liquify upon contact, most likely due to entropy of mixing, hydrogen bonding and van der Waals interactions [15]. According to theory, different DES types are described, ionic (DES I-IV) or neutral (DES V). DES-type V are created as an adequate mixture of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) that can self-associate through hydrogen-bonding interactions, which results in a strong drop in the freezing-melting point of the mixture [16]. Various interactions (such as anion exchange, weak non-covalent interactions, π-π and/or hydrogen bonding) take place, between an HBD and an HBA in various combinations and molar ratios that contribute to some of the physicochemical properties of DES [17]. DES are also viewed as cheap analogues of ionic liquids that have some important advantages, including low toxicity, high thermal stability, ease of synthesis and low cost [18]. DESs can be classified as hydrophilic or hydrophobic based on their solubility in water, which depends on the structure of the individual components of the DES. DES applications and sustainability have been improved by the use of natural components, such as terpenes. Hydrophobic DES were first reported in 2015, when they were tested for the extraction of volatile fatty acids and biomolecules, such as caffeine and vanillin, from an aquatic environment. DES used in sample preparation for pesticide determination have attracted great attention as highlighted in the review by Hu et al. [19] Werner et al. [14] have also extensively reviewed the application of DES in solid-phase (micro) extraction. Tesfaye et al. [20] prepared a group of menthol-based DES for the dispersive liquid-liquid microextraction (DLLME) of organochlorine pesticides in water and apple juice. As the HBD, either acetic acid, formic acid, or lactic acid in a 1:1 molar ratio were included. Menthol as an HBA was selected for the preparation of a set of DES for the extraction of phytocannabinoids. Sereshti et al. [21] have prepared new polymer-based deep eutectic solvents including polyethylene glycol:thymol, polyacrylic acid:menthol and polyacrylic acid:thymol as a extraction solvent for DLLME in multi-residue analysis of 16 different pesticides in water samples. Similar DES were employed by Florindo et al. [22] for the extraction of neonicotinoids from real water samples. In addition, different DESs can be applied as the coating agent or surface modifier, in combination with other materials in a synergistic way to enhance their properties [14]. One example of this approach is described in the study of López-Ruiz et al. [23]. The authors use the cellulose paper as a support for the DES to perform a sorbent-based microextraction procedure with a thymol:vanillin DES (1:1molarM ratio) in order to isolate herbicides from environmental water samples. DES as a solvent with solid supports may be a potential solution for the analysis of endocrine disrupting compounds at trace levels, as reviewed by Grau et al. [24].

The present study has aimed to develop a green sample treatment method for the determination of organophosphorus pesticide residues in water samples by means of DES incorporated in a polymeric film. Two configurations have been investigated: suspended (S)-TFME and pipette tip (PT)-TFME. The methodology for S-TFME configuration has been validated and applied with different water matrices. Preliminary results for PT-TFME are also presented.

2. Materials and methods

2.1. Reagents and solutions

The target organic compounds were the organophosphorus

pesticides MixA composed of dichlorvos, ethoprophos, disulfoton, parathion-methyl, fenchlorphos, chlorpyrifos, prothiophos, and azinphos-methyl, which were all purchased from Supelco (San Louis, MO USA). The reference material contains 2000 μg mL $^{-1}$ of each component, in hexane: acetone (9:1). The most significant properties of the compounds are shown in Table S1.

Stock standard solutions of 40 mg L^{-1} were prepared in ethyl acetate. From these stock solutions, intermediate solutions at the concentration levels needed were also prepared in ethyl acetate and replaced every two weeks. These solutions were kept at 4 °C. Standard solutions for the calibration curve were made with ethyl acetate as the solvent.

Samples for S-TFME were prepared daily in 0.01 M NaCl.

For the preparation of the films, cellulose triacetate (CTA) from Acros Organics (Geel, Belgium) was employed as the polymer. Dibutyl sabacate (DBS) was from Fluka (Buchs, Switzerland).

DES were prepared from menthol (>99.0 %) and camphor (≥99.0 %), both from Fluka (Buchs, Switzerland), and lidocaine (≥98.0 %) and dodecanoid acid (≥99.5 %) which were from Sigma (San Louis, MO USA). The properties of the DES constituents are summarized in Table 1, where the functional groups involved in hydrogen bond formation are indicated.

The solvents used were chloroform ($CHCl_3$) (≥99.8 %) from Sigma Aldrich (Steinheim, Germany); ethyl acetate (HPLC grade) from Fisher Scientific (Madrid, Spain) and acetone (HPLC grade) from PanReac Applichem (Castellar del Vallès, Spain).

All other reagents were of analytical grade. Ultrapure water was obtained from a purified system Milli-Q plus System Millipore Ibérica, S. A. (Barcelona, Spain).

2.2. Reagents and solutions

After preparing the DES according to the molar ratios shown in Table 2, the components were put into a 1.5 mL microcentrifuge tube for 15 min at 80 °C, then submitted to vortex agitation for 1 min to ensure thorough mixing [25].

2.3. Preparation and characterization of the films

The films were prepared using the solvent casting method, with CTA as the polymer (70 % w), and DES (30 % w). The procedure was as follows: 120 mg of CTA was dissolved in 15 mL of chloroform while stirring for 4 h; then 55 mg of the desired DES was added with further agitation for 1 h. After this, the solution was poured into a Petri dish (7 cm diameter), which was set horizontally, and then loosely covered. The solvent was allowed to evaporate over 24 h at room temperature, and the resulting film was then carefully peeled off the bottom (Fig. 1). The thickness of the films (measured with Digimatic micrometer, Mitutoyo) is depicted in Table 2.

Table 2
DES-film composition.

DES code	HBD:HBA molar ratio	HBD	HBA	DES film (thickness, μm)
D1	**2:1**	Dodecanoic Acid	Lidocaine	M1 (29)
D2	**1:1**	Dodecanoic Acid	Lidocaine	M2 (29)
D3	**1:2**	Dodecanoic Acid	Lidocaine	M3 (31)
D4	**1:1**	Menthol	Camphor	M4 (28)
D5	**2:1**	Menthol	Camphor	M5 (39)

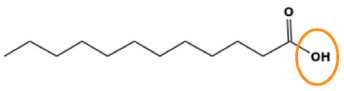
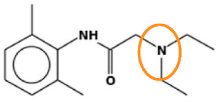
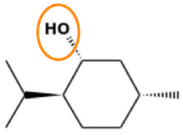
The scanning electron microscopy (SEM) images of the membranes were obtained with a field emission scanning electron microscope (FE-SEM) (Model S-4100, Hitachi, Tokyo, Japan), after placing the PIM samples on stubs and coating them with carbon (Model K950 turbo evaporator, Emitech, Montigny-le-Bretonneux, France). The image processing software Quartz PCI program (Vancouver, BC, Canada) was used to collect and process the images that were obtained.

The films were characterized with FT-IR, 13C-CPMAS solid state RMN, and Differential scanning calorimetry (DSC) techniques. FT-IR spectra were obtained with the aid of a diamond attenuated total reflectance accessory on an Agilent Cary 630 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA). For each sample, 32 scans with a resolution of 8 cm^{-1} were recorded.

^{13}C solid state NMR experiments were acquired at 12 KHz spinning rate in a BRUKER 400 MHz Ascend spectrometer equipped with an AVANCE NEO console and a 4 mm MAS VTN 1H/BB probe. Samples were fitted into 4 mm ZrO2 MAS rotors sealed with Kel-F drive caps. Chemical shifts were externally referenced to a standard adamantane sample (CH at 29.5 ppm).

Differential scanning calorimetry (DSC) analysis have been performed using an instrument manufactured by TA Instruments, model DSC Q2000. The conditions were: a temperature range from −90 °C to 100 °C; a heating rate of 10 °C/min. Nitrogen (50 ml min^{-1}) was used as

Table 1
DES components characteristics.

Name	Formula	Structure	MW (g/mol)	Melting point (C)
Dodecanoic acid	$C_{12}H_{24}O_2$		200.3178	43.2
Lidocaine	$C_{14}H_{22}N_2O$		234.3373	68.5
Camphor	$C_{10}H_{16}O$		152.2334	180.0
Menthol	$C_{10}H_{20}O$		156.2652	43.0

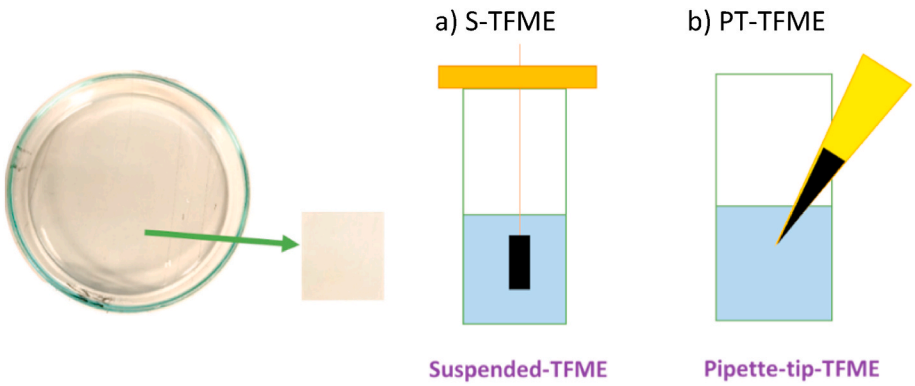


Fig. 1. Left: Film prepared with 70 % of CTA and 30 % of DES (dodecanoid acid:lidocaine 2:1). Right: scheme of the two configurations investigated: a) S-TFME; b) PT-TFME.

a purge gas. The sample was placed in a hermetic aluminium pan.

2.4. Chromatographic analysis

The chromatographic analysis was performed using a GC-MS (7820A - 5977E, Agilent Technologies, Palo Alto, CA, USA). The analytes were separated in a capillary column HP-5ms Ultra Inert (30 m × 0.25 mm I. D., film thickness 0.25 μm) from Agilent Technologies using helium as the carrier gas (1 mL min^{-1}). The following oven temperature program was applied: the initial temperature was 60 °C, maintained for 5 min, then increased from 60 °C to 150 °C at 15 °C/min and held for 4 min, and finally from 150 °C to 270 °C at 10 °C/min and held for 5 min [8].

The split/splitless injection port was equipped with an 0.65 mm ID liner and operated in splitless mode maintained at 250 °C for 1 min for automatic injection (liquid injection) and for 5 min for SPME experiments. For liquid samples, the sample injection volume was 1 μL.

The software used for analysis of the chromatographic results was MassHunter Qualitative Analysis; ions for quantification and retention times are shown in Table S1.

2.5. Extraction procedures

2.5.1. Exhaustive extraction

To select the most convenient DES composition, exhaustive extraction for the OPPs was evaluated. The extraction procedure was performed with a 4 cm^2 piece of film contacted with 20 mL of 0.01 M NaCl solution spiked at 50 μg L^{-1} with the OPPs mixture. A rotatory agitator was used, and agitation was maintained for a time lapse of 6 h at 50 rpm.

For the determination of the compounds in the aqueous solution before and after the extraction experiment, solid-phase microextraction was used. The conditions were: a PDMS/DVB coated fibre and direct immersion extraction over a 30 min period at room temperature using 7 mL solution. The chromatographic conditions were as reported above (Section 1.4).

The extraction efficiency (EE%) was measured from the SPME results, and was defined using equation (1):

$$EE\% = \left(1 - \frac{A_f}{A_i}\right) \times 100 \quad (1)$$

where A_i is the SPME-peak area of the compound at t = 0 min and A_f is the SPME-peak area after 6 h contact time.

2.6. Elution procedure

After the extraction step, the piece of film was removed from the solution, washed with ultrapure water, gently dried using a piece of paper, and prepared for the elution. The elution conditions were adapted from Vera et al. [7]. Briefly, 2 mL of ethyl acetate were added and 15 min ultrasound-assisted (US) extraction was applied. The organic extract was analysed in the GC-MS. The amount of compound eluted was obtained from a calibration curve prepared in ethyl acetate containing the matrix compounds present in the film. Briefly, a piece of membrane was contacted with the NaCl solution and subsequently eluted following the procedure described above. Afterwards, an appropriate volume of the stock solution of the target compounds was added to the 2 mL ethyl acetate. The matrix-matched calibration standards were prepared in a range from 50 to 1000 μg L^{-1}.

Elution efficiency (RR%) was calculated using equation (2)

$$RR\% = \frac{m_{el}}{m_{ext}} \times 100 \quad (2)$$

where m_{el} is the mass of eluted compound and m_{ext} the mass of the compound present in film after extraction (calculated by mass balance using the extraction efficiency).

2.7. Suspended-thin film microextraction procedure

Once the film composition had been evaluated, the selected DES film was used for S-TFME with the following procedure. A piece of the film (1 cm × 2 cm) was placed in contact with the solution (10 mL) for 1 h under magnetic stirring at room temperature. A stainless-steel rod attached to the cap of the vial was used as support (see Fig. 1a). The film was eluted in an ultrasound bath for 15 min using 1 mL ethyl acetate. Then, 1 μL was taken and injected in the GC-MS and area values were obtained.

The S-TFME procedure was applied to standard solutions at different analyte concentrations (1–166 μg L^{-1} for the OPPs), prepared in 0.01 M NaCl, and to spiked real waters (see section 2.9).

2.8. Pipette tip thin-film microextraction

The PT format was evaluated for on-site application (Fig. 1b). The pipette tip was prepared in a similar way as for micro-solid phase extraction (μ-SPE), as described by Seidi [10]. A piece of the film (2.9

cm^2) was placed inside a pipette tip with a capacity of 1 mL. A series of experiments were carried out to optimize the PT-TFME procedure. Extractions were performed using 10–20 mL of 0.01 M NaCl solution spiked with the analytes at a concentration of 200 μg L^{-1} for chlorpyrifos (CPS), which was selected as a model OPP pesticide. Different extraction and desorption cycles were tested (each cycle comprising one withdraw/release step). During extraction, 1 mL was withdrawn for each cycle and released again into the solution, while the bulk solution was homogenised using magnetic agitation. The desorption step was performed using 1 mL of ethyl acetate as previously indicated for S-TFME or 200 μL (for desorption cycles). Preliminary tests were carried out with a film composed of CTA (70 % w) and the plasticizer dibutyl sebacate (30 % w).

Once the experimental parameters had been fixed, the methodology was tested for CTA:DES film (M1) at different chlorpyrifos concentrations in a 20 mL solution and in a 20 L tank containing spiked tap water to simulate on-site conditions. In the tank, agitation with blades was set at 200 rpm. Finally, PT-TFME was also tested for the OPP mix.

2.9. Real water samples

Two distinct types of water samples were used: the Osor River (collected in November 2021) and a sample of the secondary tank of the wastewater treatment plant (WWTP) in Quart, Girona (Catalonia, Spain) that mainly receives domestic wastewater. The absence of the target compounds was verified. The physico-chemical characteristics of the water samples are collected in Table 3.

Tap water from the municipal network of Girona was used for some experiments (see https://laboratori.catsa.cat/es/inici.html for water characteristics). Water was deaired for one day in order to eliminate free chlorine.

Unless otherwise indicated, all the experiments described in this study were run in triplicate.

Table 3
Physico-chemical characteristics of water samples tested.

	Sampling point	
	Osor River	WWTP effluent
pH	8.19	7.22
Cond. (μS cm^{-1})	135	617
TAC mg HCO_3 L^{-1}	51.283	98.627
NO_3^- mg L^{-1}	3.648	43.457
PO_4^{3-} mg L^{-1}	0.546	0.411
Cl^- mg L^{-1}	11.379	175.850
SO_4^{2-} mg L^{-1}	23.670	75.140
F^- mg L^{-1}	0.241	0.315
Na^+ mg L^{-1}	12.195	126.298
K^+ mg L^{-1}	1.427	21.663
Mg^{2+} mg L^{-1}	4.834	10.126
Ca^{2+} mg L^{-1}	23.879	62.521
TOC mg C L^{-1}	2.362	9.613

3. Results and discussion

3.1. Characterization of the films

The new films containing CTA and DES were successfully prepared for the first time. The material is transparent, mechanically stable and easy to manipulate. Scanning electric microscopy was used to verify the film morphology and porosity (Fig. 2). The absence of micropores, as reported for polymeric membranes prepared with CTA and a plasticizer, was confirmed [8].

The different films were characterized by FT-IR, solid state ^{13}C NMR, and DSC techniques. In Fig. S1, FT-IR spectra are shown, where the characteristic band of the DES components, the prepared DES, the polymer CTA, and the film M1 can be observed. The lidocaine spectrum shows the prominent peaks at 3243 cm^{-1} (N–H stretching), at 2965 and 2797 cm^{-1} (aliphatic C–H stretching), at 1661 cm^{-1} (C═O stretching), and at 1593 cm^{-1} (aromatic C═C stretching). In the dodecanoic acid spectrum, a broad band starting at 3200 cm^{-1} (O H stretching) overlapping with the peaks at 2912 and 2845 cm^{-1} (aliphatic CH) can be observed. A band at 1690 cm^{-1} (C═O stretching) is also shown. When compared to the DES, the most affected band is that at 3243 cm^{-1}, which is shifted to lower wavenumber (3201 cm^{-1}), with an evident decrease of intensity attributed to the hydrogen bonding. The FT-IR spectrum of the film M1 shows the typical bands of the CTA polymer: the absorption peak at 1736 cm^{-1} corresponding to the C═O stretching band, and the peak at 1031 cm^{-1} attributed to the C–*O*–C group. Characteristics bands of the DES components, such as the band at 3201 cm^{-1} (lidocaine) and the band at 2919 cm^{-1} (dodecanoic acid) for the C–H from dodecanoic acid, cannot be observed in the film or are very weak.

^{13}C NMR of pure components and M1 film were measured to deepen our insight of the interactions between the polymer and the DES present in the sample. Van Osch et al. obtained ^{13}C NMR spectra of different DES to investigate whether a chemical reaction occurred between the two components of the DES leading to extra peaks. From the comparison between the experimental molar ratios and the theoretical expectations, they concluded that no reaction took place between the components. In the spectrum of CTA alone (Fig. 3), the signals observed and the chemical shifts (glucosidic ring, methyl and carbonyl carbons) correspond to those described [26]. The chemical shifts attributed to CTA remain unchanged when the DES is added to the polymer (film M1). Additional less intense signals in the M1 spectrum can be attributed to the DES components, particularly the aromatic C signal of lidocaine at 130–140 ppm and the signal of C_{CH3} at 10–15 ppm. The shifts observed when comparing the M1 film spectrum and the spectra for the pure components are due to the different environment that the CTA polymer provides in the M1 film.

DSC was used to get information on the interaction of DES D1 and CTA in film M1. In Fig. S2 the results are shown, together with the results of a film containing only CTA. In the analyses conducted on pure samples of lidocaine and dodecanoic acid, a single peak corresponding to the fusion of these products was observed (69.1C for lidocaine and

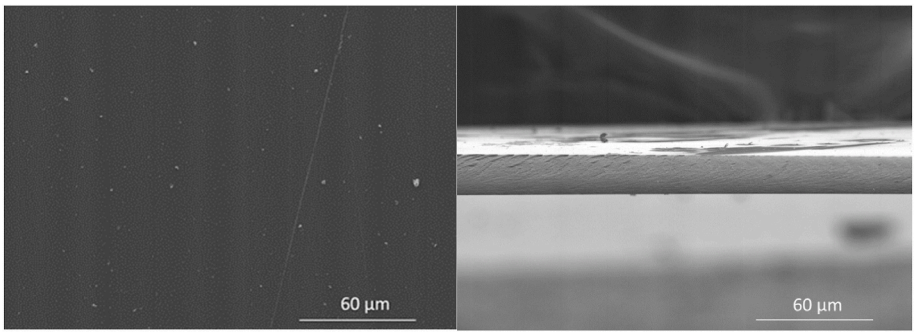


Fig. 2. SEM images of film M1 (CTA:DES, 70:30 %): left, surface; right cross-section.

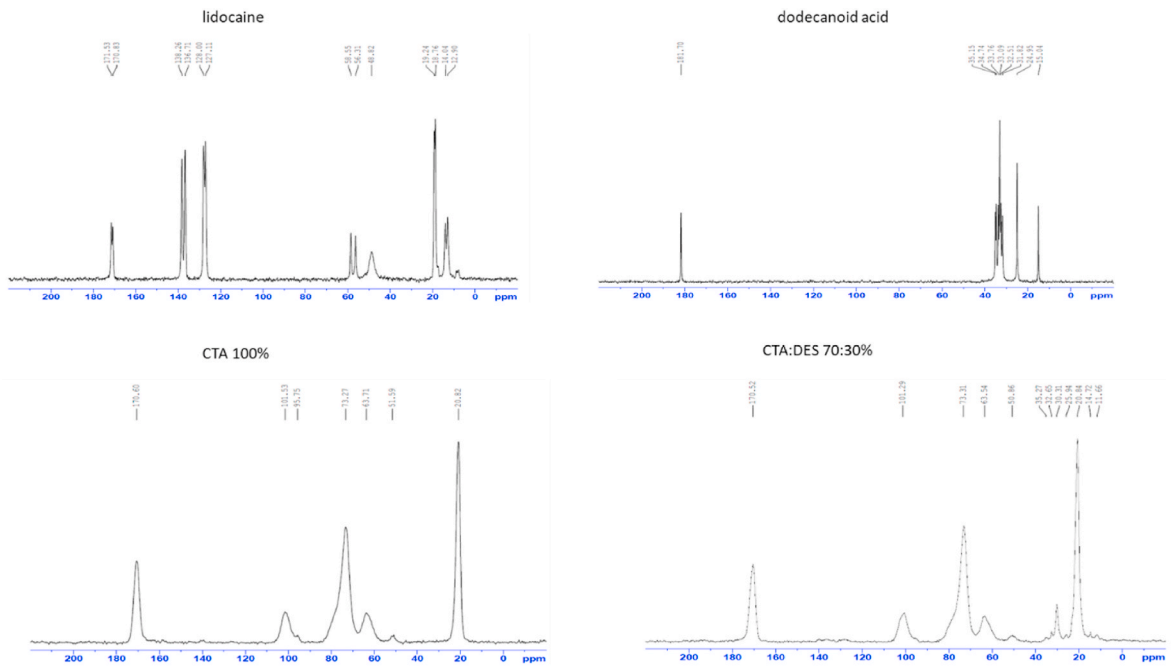


Fig. 3. 13C NMR spectra of lidocaine, dodecanoic acid, CTA, and film M1 (CTA:DES, 70:30 %).

46.2C for dodecanoic acid).

In the eutectic mixture (Figure S2 a)), a melting peak is shown at −19.3C, followed by an exothermic peak at −0.51 °C, probably due to the recrystallization of the product into a new phase, and finally, the melting of this new phase is observed with a peak at 17.43C. There is no presence of peaks corresponding to the pure components.

In the film M1 (Figure S2 d)), there is indeed a clear endothermic peak at 270C, which would be the fusion of the CTA. This peak appears at 290C in pure CTA (Figure S2 b). The fusion enthalpy is 14 J/g; considering that the membrane contains 70 % CTA, the fusion enthalpy per gram of CTA would be 20 J/g, which is similar to the 19 J/g obtained with CTA100 %. Other peaks at lower temperatures in Figure S2 d) dare more complicated to interpret. There is an endothermic peak at 132.3 °C that could relate to a phase transformation in the material. In Figure S2 c), the endothermic peaks associated to the pure DES components are not observed, neither the peaks of the two polymorphs of the eutectic solvent. This indicates the strong interaction of the mixture with the CTA polymer.

3.2. Extraction performance

A film with a CTA:DES ratio composition of 70:30 (%, w/w) was used to evaluate the extraction efficiency for OPPs in exhaustive extraction (6 h). After some preliminary trials, six out of eight OPPs present in the mix were taken for the study. Disulfoton and azinphos-methyl were discarded due to lack of stability, and to poor response in the GC-MS, respectively. As we can observe in Fig. 4, as a general trend, parathion-methyl, fenchlorphos, chlorpyriphos, and prothiophos are the compounds that present greater affinities for the films, with EE > 85 %. M1, M3 and M5 are the films that show the best performance, although ethoprophos could not be measured in the water solutions after extraction for M1 and M2 due to the overlapping of its chromatographic peak with that of dodecanoic acid. A blank experiment with a piece of film made of 100 % CTA resulted in a 0 % extraction efficiency for the target compounds.

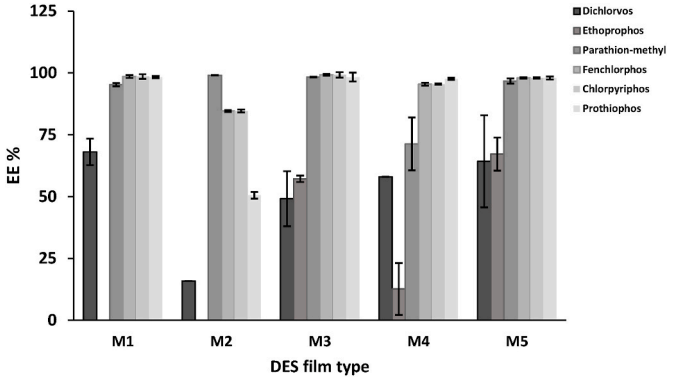


Fig. 4. Exhaustive extraction evaluation of the different DES films.

3.3. Elution

The next step was to evaluate the elution efficiency. TFME usually requires a solvent for the elution of the extracted compounds. Based on our previous results for chlorpyrifos, ethyl acetate was selected.

The results are presented in Fig. 5. Two mL of solvent were used and the solution was injected into the GC-MS without a further evaporation

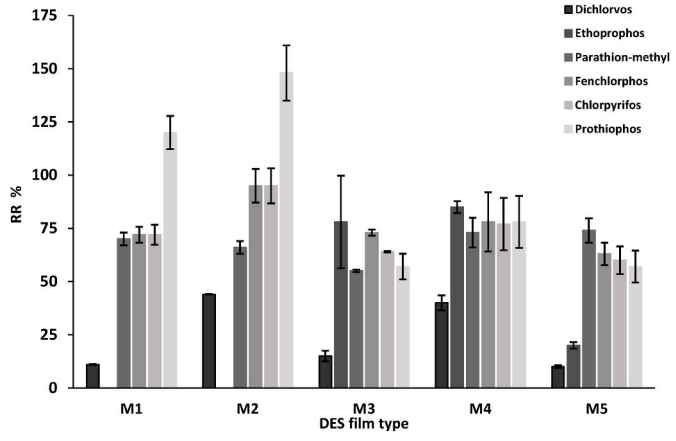


Fig. 5. Elution efficiency for the different films. Two mL of ethyl acetate was used for the elution.

step. The elution efficiency was determined according to equation (2).

For M1 and M2, the elution efficiency for ethoprophos could not be calculated, as has been stated earlier. The elution step was satisfactory for most of the compounds. For dichlorvos lower RR, in the range 10–44 % were achieved. Considering the extraction and elution results all together, the M1 film was selected for further application in the microextraction of the selected OPPs, using the S-TFME and PT-TFME configurations.

3.4. Application of DES-films for S-TFME

After the M1 film was selected, S-TFME was performed. The microextraction procedure followed is similar to SPME (section 2.7); in this case, a piece of film is selected instead the SPME fibre. The film was suspended (immersed) in the solution, under agitation; 1-h extraction time was selected as a compromise between sensitivity and sample throughput. After the extraction, solvent elution was applied as indicated in the experimental part, and the organic extract was analysed by GC-MS. In Fig. 6, a chromatogram is shown for a solution containing 50 μg L^{-1} OPPs after S-TFME and elution.

The peaks corresponding to the two DES components can be observed in the chromatogram, dodecanoic acid at t = 16.476 min and lidocaine at t = 21.406 min. Due to the back extraction of the DES components, the films can only be used once.

3.5. Figures of merit of the proposed S-TFME method

For the validation of the method, calibration curves were measured in the range 5–166 μg L^{-1} (Fig. S3). The linear response in the concentration range studied was verified.

In Table 4, the quality parameters of the method are summarized. Determination coefficients (R^2) were >0.992 for all the compounds except for parathion-methyl. LODs and LOQs were determined as in equation (3):

$$LOD = \frac{k \times SD}{slope} \quad (3)$$

where SD is the standard deviation (n = 5) calculated from the standard solution at the lowest concentration level (5 μg L^{-1}), and k = 3 is used for LOD and k = 10 for LOQ calculation.

LODs in the range 0.4–1.0 μg L^{-1} were obtained except in the case of ethoprophos (1.3 μg L^{-1}). These values are similar to those reported in López-Ruiz et al. [23] for some selected triazine herbicides, using deep eutectic solvent coated paper as sorptive phase, elution with 1 mL methanol and GC-MS determination. Moreover, in DLLME for organochlorine pesticides, LODs are one order magnitude lower, using smaller volumes of extraction solvent [20,21]. In spite of the higher LODs found in our study, the easy manipulation of the extraction phase when microextraction is performed with a film is an important advantageous issue compared to DLLME.

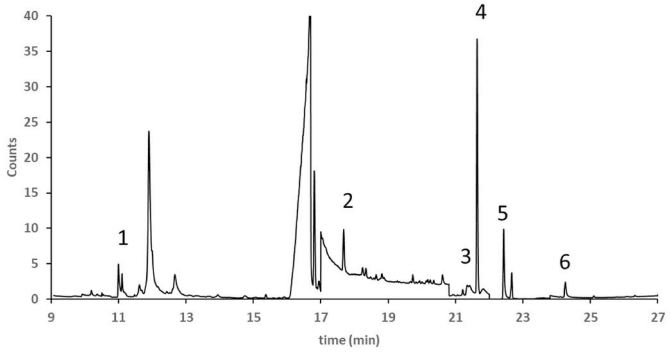


Fig. 6. Chromatogram (SIM mode) for a standard solution at 50 μg L^{-1} after S-TFME and elution. 1. dichlorvos, 2. ethoprophos, 3. parathion-methyl, 4. fenchlorphos, 5. chlorpyriphos, and 6. prothiophos.

Table 4
Quality parameters of the S-TFME method.

Compound	*LOD (μg L^{-1})*	*LOQ (μg L^{-1})*	Equation for the linear model	R^2	*RSD % n = 5* (5 μg L−1)	*RSD % n = 3* (96 μg L−1)
Dichlorvos	*1.0*	*3.3*	*y = 36.434x – 4.1191*	*0.992*	*6*	*11*
Ethoprophos	*1.3*	*4.3*	*y = 119.34x – 639.69*	*0.996*	*14*	*7*
Parathion-methyl	*0.4*	*1.3*	*y = 360.91x – 3608.2*	*0.981*	*7*	*3*
Fenchlorphos	*1.0*	*3.3*	*y = 1095.4x – 1077.8*	*0.992*	*7*	*14*
Chlorpyrifos	*0.7*	*2.3*	*y = 327.17x – 1040.7*	*0.997*	*6*	*14*
Prothiophos	*1.0*	*3.3*	*y = 184.2x – 1149.7*	*0.996*	*11*	*6*

Altogether, the obtained quality parameters were considered promising for the environmental application of the developed method provided that more sensitive equipment is used for example high resolution mass spectrometry or tandem mass spectrometry as instrumental techniques.

Table 5
Comparison of the different matrices and spiked at 50 μg L^{-1} for the OPPs compounds (n = 3). Results given as recovery (R %) and standard deviation in brackets.

Analyte	WTTP effluent	Osor
	R %	R %
Dichlorvos	69 (14)	86 (14)
Ethoprophos	69 (17)	78 (13)
Fenchlorphos	103 (7)	101 (9)
Chlorpyrifos	72 (7)	64 (6)
Protiophos	163 (34)	132 (7)

Intra-day precision, expressed as the relative standard deviation (RSD %), was measured at two levels, 5 and 96 μg L^{-1}. RSD% lower than 14 were found, which are considered suitable taking into account the concentration level [3].

3.6. Application to real water samples

The method was tested using different water matrices from the Osor River and the effluent of the Quart wastewater treatment plant. Both water samples were spiked at 50 μg L^{-1}. Previous analysis of the water samples did not reveal the presence of any of the compounds. In Table 5, the recovery values [27] for the two samples are presented.

As can be observed from the table, the compounds exhibited different behaviours. Satisfactory recoveries were obtained for fenchlorphos in both matrices. Dichlorvos, ethoprophos and chlorpyrifos show absolute recoveries ranging from 64 to 86 % in Osor River water, and 79-62 % in wastewater sample. These values are in the range accepted for the ug L^{-1} concentration level [28]. For protiophos, recoveries were 132 % and 163 % for Osor River and wastewater sample, respectively, which indicate the presence of some interference or matrix effect affecting the analytical response.

For parathion-methyl, values under 10 % were found (not shown in the table). Considering that microextraction techniques are not equilibrium techniques, these results for parathion-methyl can be attributed to the interaction of the target analytes with components present in the complex water matrix. When this occurs, the bound compounds cannot be extracted by the S-TFME method as observed in Roy et al. [29] where a low response was observed due to the binding of drugs to plasma proteins.

3.7. Pipette tip microextraction

Solid sorbents placed inside a disposable pipette tip are usually employed for pipette tip microextraction. This configuration presents some advantages, such as the need for small solvent elution volumes and ease of operation, which makes it suitable for on-site application. To the best of our knowledge, this is the first time that a thin film comprising a polymeric membrane containing DES has been applied for PT-TFME. The PT-LPME configuration requires the evaluation of different parameters: number of extractions, number of extraction cycles, the volume of the sample, and the volume of the solvent used for elution. Preliminary experiments were performed with a film containing 70 % CTA: 30 % DBS (w/w), which had previously been used successfully for the microextraction of chlorpyrifos and other organic pollutants [8].

The number of extraction cycles was the first factor evaluated taking a 10 mL sample solution. Five, ten, and twenty extraction cycles were compared, as can be seen in Fig. 7. Elution was carried out with one mL ethyl acetate after removing the film on the outside of the pipette tip.

As can be seen in the figure, no significant differences were obtained in peak areas. However, five extraction cycles result in higher repeatability in terms of relative standard deviation (RSD). Since less time is required to carry out five cycles, this value was selected.

The following experiments were carried out using the CTA:DES M1 film. Firstly, the elution was investigated. The PT set-up allowed the elution to be performed using different withdrawal/release cycles (elution cycles) with 200 μL ethyl acetate, instead of the US elution (one mL solvent) in a separate step outside the pipette tip. In Fig. 8, both procedures are evaluated. As can be seen, the 200 μL elution in five cycles provides satisfactory results, increasing the simplicity of the method.

Finally, the sample volume and the number of elution cycles were also tested. Ten, twenty, and forty mL samples were compared with no statistical differences being found in the responses obtained. In the case of the number of withdrawal and release cycles for the elution, using 200 μL ethyl acetate, better response was observed for 10 cycles (Fig. S4). Therefore, the final conditions for the PT method were 20 mL samples, 5 extraction cycles and 10 elution cycles.

The response of the method at different concentration levels of chlorpyrifos was then tested. The results are plotted in Fig. 9.

Given the satisfactory results obtained, the experiment was also performed in a 20 L tank filled with tap water from the Girona municipal network. This set-up was designed to simulate natural conditions for environmental water bodies. The microextraction was performed at 50 μg L^{-1} and 100 μg L^{-1} of chlorpyrifos and a distinct response (in terms of peak area) for the two concentration levels was obtained that corresponds to spiked concentration ratio. These preliminary results encourage us to continue pursuing our investigations in this area.

Finally, the optimized PT-TFME was tested for OPP mix using 20 mL 0.01 M NaCl solution spiked at 50 μg L^{-1}. The PT allowed the detection of all the OPP compounds except ethoprophos (Fig. 10).

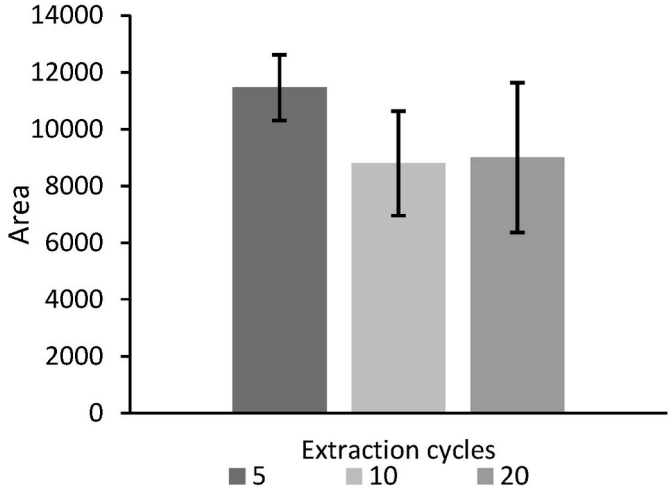


Fig. 7. Peak area for chlorpyrifos for different extraction cycles. Film: CTA:DBS 70:30 % (w/w). Sample: 0.01 M NaCl solution (10 mL) spiked at 200 μg L^{-1} de CPS. Elution: 1 mL ethyl acetate, 15 min US.

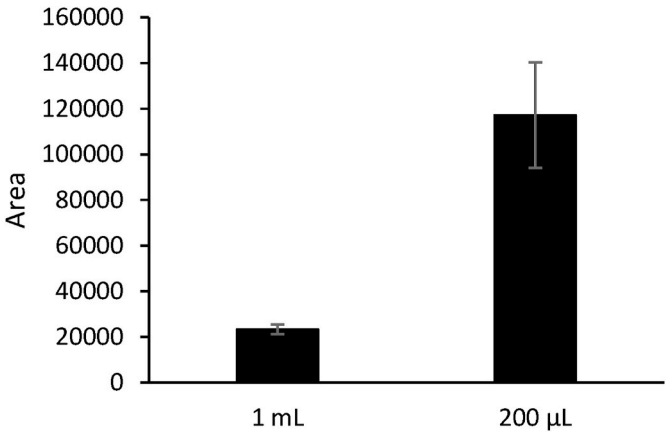


Fig. 8. Response for the different elution procedures tested: 1 mL ethyl acetate with 15 min US and 200 μL ethyl acetate for 5 cycles. Sample: 0.01 M NaCl spiked at 200 μg L^{-1} CPS. Extraction, 5 cycles. Sample volume, 10 mL.

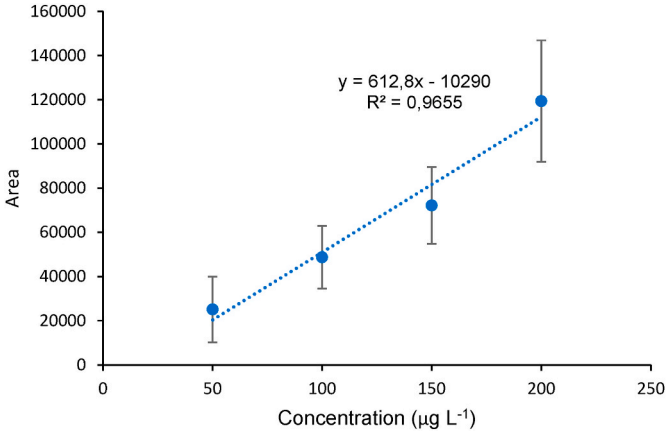


Fig. 9. Calibration curve for the PT-TFME. Extraction, 5 cycles. Elution, 10 cycles (200 μL ethyl acetate). Sample solution, 20 mL.

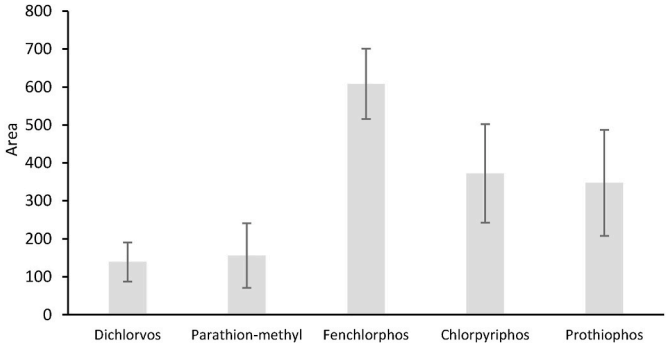


Fig. 10. PT-TFME for OPPs (50 μg L^{-1}). Extraction, 5 cycles. Elution, 1 mL ethyl acetate with 15 min US.

4. Conclusions

In this work, we have developed an efficient DES-based TFME method for the extraction and preconcentration of OPPs from water samples before analysis by GC–MS. The films were prepared by solvent casting using CTA as the polymer. Among the DESs that were prepared, containing lidocaine or menthol as HBA and dodecanoid acid or camphor as HBD, in different molar ratios, the dodecanoic acid:lidocaine mixture (molar ratio 2:1) was selected on the basis of its extraction efficiency and the elution results. With regard to the characterization of the SEM films, FT-IR, ^{13}C solid state RMN and DSC techniques were used. For the suspended film set-up, quality parameters of the analytical method were calculated, i.e., a linear range between 1 and 166 ng mL^{-1}, LODs between 0.4 and 1.3 ng mL^{-1}, and satisfactory precision (RSD) from 3 to 14 %. The applicability of the method was also assessed by analysing OPPs from different water samples (river water and effluent from a WWTP), and the influence of a matrix effect was discussed. Preliminary results using the film placed inside a disposable pipette allowed the number of extractions (five) and elution cycles (ten) to be established, allowing it to be used for on-site monitoring purposes.

In conclusion, the DES-based TFME method developed here is rapid, simple, environmentally friendly, and feasible for the determination of trace-level OPPs in water in their free forms.

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5. Institutional review Board statement

Not applicable.

6. Informed consent statement

Not applicable.

CRediT authorship contribution statement

Ivonne Quintanilla: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Clàudia Fontàs:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Enriqueta Anticó:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2024.342940.

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