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RESEARCH PAPER

Magnetic ionic liquid-based dispersive liquid-liquid microextraction technique for preconcentration and ultra-trace determination of Cd in honey

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Abstract

A simple, highly efficient, batch, and centrifuge-less dispersive liquid-liquid microextraction method based on a magnetic ionic liquid (MIL-DLLME) and electrothermal atomic absorption spectrometry (ETAAS) detection was developed for ultra-trace Cd determination in honey. Initially, Cd(II) was chelated with ammonium diethyldithiophosphate (DDTP) at pH 0.5 followed by its extraction with the MIL trihexyl(tetradecyl)phosphonium tetrachloroferrate(III) ($[P_{6,6,6,14}]FeCl_4$) and acetonitrile as dispersant. The MIL phase containing the analyte was separated from the aqueous phase using only a magnet. A back-extraction procedure was applied to recover Cd from the MIL phase using diluted HNO_3 and this solution was directly injected into the graphite furnace of ETAAS instrument. An extraction efficiency of 93% and a sensitivity enhancement factor of 112 were obtained under optimal experimental conditions. The detection limit (LOD) was 0.4 ng L^{-1} Cd, while the relative standard deviation (RSD) was 3.8% (at 2 μg L^{-1} Cd and $n = 10$), calculated from the peak height of absorbance signals. This work reports the first application of the MIL $[P_{6,6,6,14}]FeCl_4$ along with the DLLME technique for the successful determination of Cd at trace levels in different honey samples.

Keywords Magnetic ionic liquids · Microextraction · Preconcentration · Cadmium · Honey

Introduction

Cadmium is a highly toxic element that occurs normally in nature at low concentrations. However, industrial activities can contribute to increase the abundance of Cd in the environment, mainly due to contaminated wastewaters and the combustion of coals and oils, among others [1]. The accumulation of Cd in humans can provoke hypertension, osteoporosis, lung, liver, and kidney disorders [2]. Considering that the FAO/WHO Joint Committee on Food Additives establishes a maximum tolerable daily intake of Cd of 1.0–1.2 μg kg^{-1} body mass [3], it is necessary to regulate its concentration in all kind of foods and other types of samples that could represent exposure routes. It has been demonstrated that Cd can be found in honey as a result of bees carrying the metal from nectar flowers to honey [4]. Therefore, the control of Cd concentrations in honey and foods incorporating honey in their formulation acquires special attention not only for adults but also for infants and young children who are frequent consumers of honey and derived products. In this way, the MERCOSUR regulation in South America has established a maximum permissible limit for Cd in honey of only 0.1 μg g^{-1} [5]. In order to reach accurate and sensitive results in the determination of Cd in honey at these low concentration levels, a preconcentration step is often required before the detection of the metal by most instrumental detection techniques.

Up to date, different extraction techniques have been reported for the preconcentration of Cd in honey samples, including solid phase extraction (SPE) [6, 7], cloud point

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extraction (CPE) [8], and liquid-liquid microextraction (LLME) [9]. Generally, classical microextraction techniques use volatile organic compounds (VOCs) as extraction agents that are not in good agreement with environmental preservation. In order to decrease the use of VOCs, ionic liquids (ILs) have been proposed as valuable alternatives. Ionic liquids (ILs) are organic molten salts with low melting points, characterized by a negligible vapor pressure, thermal stability at high temperatures, and specificity towards desirable ions [10]. Ionic liquids have proved to be successful extraction agents in LLME or can be combined with SPE preconcentration techniques for the determination of Cd in several food samples [11, 12]. However, in the majority of the reported microextraction procedures, the separation of the sedimented phase from the liquid phase is performed by centrifugation, which causes a negative effect in the cost and frequency of analysis. Likewise, LLME techniques require ILs denser than water in order to achieve an effective separation of the phases after analyte extraction. These drawbacks could be fully solved by using a more recent generation of ILs named magnetic ILs (MILs), which offer outstanding characteristics including good capacity for the extraction of inorganic and organic analytes and also magnetic properties [13]. These properties give to MILs the possibility of separating the sedimented phase from the upper phase using simply a magnet, thus avoiding the classical time-consuming centrifugation step involved in most LLME procedures. The magnetic trihexyl(tetradecyl)phosphonium IL have been synthesized, characterized, and applied in LLME for the extraction of organic compounds and biomolecules [4, 14, 15]. Nevertheless, this MIL has been poorly explored as extractant agent of metal ions for both analytical and technological developments [16].

The aim of the present work was the development of a batch and centrifuge-less dispersive LLME (DLLME) technique based on the application of the MIL trihexyl(tetradecyl)phosphonium tetrachloroferrate ($[P_{6,6,6,14}]FeCl_4$) for the efficient extraction and preconcentration of Cd. The element was initially chelated with ammonium diethylditiophosphate (DDTP) reagent. The optimization of all experimental variables influencing the microextraction, including pH, complexing agent, volume of MILs, stirring time, sample volume, and back-extraction conditions was performed. The proposed method was successfully applied for the determination of Cd at ultra-trace levels in honey samples by the MIL-DLLME technique and electrothermal atomic absorption spectrometry (ETAAS) detection. Matrix effects and potential interfering species caused by the MIL and honey samples were carefully studied for accurate determination of Cd. To the best of the authors' knowledge, a DLLME method using $[P_{6,6,6,14}]FeCl_4$ is reported for the first time in the analytical field for Cd preconcentration and determination.

Experimental

Instrumentation

The experiments were performed using a Perkin Elmer Model 5100ZL atomic absorption spectrometer (Uberlingen, Germany) equipped with a transversely heated graphite atomizer and a Zeeman-effect background correction system. A Cd electrodeless discharge lamp (PerkinElmer, Norwalk, CT, USA) operated at a current of 170 mA and a wavelength of 228.8 nm with a spectral band width of 0.7 nm was used. The instrumental parameters and temperature program of the graphite atomizer are shown in Table 1. A vortex model Bio Vortex B1 (Boeco, Hamburg, Germany) was used for mixing the reagents. A Horiba F-51 pH meter (Kyoto, Japan) was utilized for pH determinations. A rotary evaporator (Senco, Shangai, China) was used for the synthesis of the MILs. A magnetic stirrer (Thorbell, Santa Fe, Argentina), a drying stove (San Jorge, Buenos Aires Argentina), an ultrasound bath (Testlab, Buenos Aires, Argentina), and a neodymium magnet (1.17 T magnetic field) were also utilized.

Reagents

All the reagents were of analytical grade and the presence of Cd was not detected within the working range. A 1000-mg L^{-1} Cd(II) stock standard solution was prepared dissolving $Cd(NO_3)_2$ (Merck, Darmstadt, Germany) in 0.1 mol L^{-1} HNO_3 (Merck). Lower concentrations were prepared by diluting the previous solution with 0.1 mol L^{-1} HNO_3. A 10^{-3} mol L^{-1} DDTP (Sigma-Aldrich, Milwaukee, USA) solution was prepared in methanol (Merck). Lower concentrations were prepared by serial dilutions with methanol. For chemical modification in ETAAS, 150 mg L^{-1} $Mg(NO_3)_2$ (Merck) and 2500 mg L^{-1} $NH_4H_2PO_4$ (Merck) solutions were used. Hydrochloric acid (37%) (Ultrapure grade, Figmay, Córdoba, Argentina), acetonitrile (99.9%), methanol (99.9%), and chloroform (99.9%) from Merck were also used. A 3% (*w*/*v*) NaCl (99.9%, Merck) solution and $NaNO_3$ (99.0%, Sigma-Aldrich) were prepared to adjust the ionic strength. CYPHOS®IL 101 (trihexyl(tetradecyl)phosphonium chloride) was from CYTEC Industries Inc. (NJ, USA). Iron(III) chloride hexahydrate (98%, Sigma-Aldrich) and iron(II) chloride tetrahydrate (98%, Sigma-Aldrich) were used for the synthesis of MILs. Ultrapure water (18 MΩ cm) was obtained from a Millipore Continental Water System

Table 1 ETAAS instrumental conditions for Cd determination in honey

Instrumental conditions				
Wavelength			228.8 nm	
Spectral band width			0.7 nm	
EDL lamp current			170 mA	
Matrix modifier			50 μg $NH_4H_2PO_4$ 3 μg Mg	
Graphite furnace temperature program				
Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying 1	110	1	30	250
Drying 2	130	10	30	250
Pyrolysis	500	20	20	250
Atomization	1400	0	4	–
Cleaning	2400	1	2	250

(Bedford, MA, USA). All bottles destined for storing standard solutions and the glassware were washed in 10% (*v*/*v*) HNO_3 for 24 h and later rinsed with ultrapure water.

Preparation of MILs

The MILs were synthesized following a method previously reported. The MIL $[P_{6,6,6,14}]FeCl_4$ and $[P_{6,6,6,14}]_2FeCl_4$ were synthesized based on the method reported by Del Sesto et al. [17]. Basically, equimolar amounts of $FeCl_3{\cdot}6H_2O$ or $FeCl_2{\cdot}4H_2O$ were mixed with a solution 50% (*w*/*v*) of CYPHOS IL 101 dissolved in chloroform. They were left to react for 24 h at room temperature under constant agitation. Afterwards, the excess of chloroform was removed by a rotary evaporator and the product obtained was dried overnight at 50 °C.

On the other hand, the 1-octyl-3methylimidazolium chloride ($[C_8mim]Cl$) was synthesized based on the method reported by Wang et al. [18]. An equimolar mixture of $[C_8mim]Cl$ and $FeCl_3{\cdot}6H_2O$ was prepared in methanol and kept under nitrogen atmosphere for 4 h. The solvent was then evaporated and the product washed with deionized water to remove the excess of $FeCl_3$ remaining in the synthesized MIL. Finally, the MIL was dried under vacuum at 90 °C for 48 h.

All MILs were characterized by observation of the spectra obtained by Fourier transformed infrared spectroscopy (FTIR). Main functional groups were confirmed in each of the MIL synthesized according to the abovementioned procedures. The spectral information was also compared with that reported by other authors for the same type of MILs [17].

Sample collection and conditioning

The honey samples were purchased at local markets of Mendoza City (Argentina). All analyzed samples were produced in different regions of Mendoza that are placed far from industrial plants or intense anthropogenic activities. The honey samples were stored at 4 °C in absence of light until analysis. In order to decrease the viscosity of the samples, solutions at 1% (*w*/*v*) honey were prepared in 1% (*v*/*v*) HNO_3 before analysis.

Dispersive liquid-liquid microextraction procedure based on MILs

For the development of the microextraction procedure, a volume of 30 mL of sample solution (1% (*w*/*v*) honey in 1% (*v*/*v*) HNO_3), or a 2-μg L^{-1} Cd(II) standard solution (for optimization), was placed in a 50-mL graduated centrifuge tube along with 60 μL of 1×10^{-3} mol L^{-1} DDTP and 420 μL of 37% (*v*/*v*) HCl to adjust the pH at 0.5. The mixture was shaken for 1 min and left to stand for 2 min to ensure the complete formation of the Cd-DDTP complex. Then, 100 μL of MIL extraction phase (50% (*w*/*v*) of MIL prepared in chloroform) and 300 μL of acetonitrile (dispersant) were added. The mixture was shaken for 15 min with a vortex stirrer. Subsequently, a magnet was used to separate the MIL phase from the aqueous phase. Finally, 500 μL of 1% (*v*/*v*) HNO_3 were added to back-extract the analyte from the MIL phase, sonicating the mixture during 15 min. A volume of 20 μL of the phase containing the analyte was injected into the graphite furnace of ETAAS instrument for Cd determination (conditions as shown in Table 1). Calibration was performed against aqueous standards and blank solutions.

Results and discussion

Preliminary studies on Cd determination by ETAAS in the MIL matrix

The primary aim of this work was to perform the extraction of Cd with a MIL followed by the determination of this element with ETAAS in the presence of the organic and inorganic matrix added by this type of extractant into the graphite furnace. Therefore, different experiments were performed to evaluate the feasibility of Cd determination in the presence of complex matrix of MILs. Due to the high viscosity of MILs, it was necessary to dissolve them in an appropriate solvent (50 μL methanol) before its injection into the graphite furnace to overcome drawbacks in the reproducibility. The selected solvent was methanol because it was observed as appropriate for solubilization of MILs in this media. Unfortunately, it was observed that the detection of Cd was negatively affected by the MILs matrix, with poorly reproducible analyte signals and a remarkable high background signal. Experiments were performed to explain this behavior by injecting 20 μL of 2 μg L^{-1} Cd(II) standard solution in the presence of different amount of Fe. The results showed that Fe strongly interfered on Cd signal from 2 μg Fe (Fig. 1). This effect has been assigned to the spectral interference caused by Fe over Cd in AAS measurements [19]. This mass of Fe was significantly lower than the contribution made by injecting the MIL extractant phase (i.e., 4.11 μg Fe in 100 μL of $[P_{6,6,6,14}]FeCl_4$ extraction phase). Thus, the high concentrations of Fe present in the MILs were the main cause of severe interference in the determination of Cd by ETAAS. Moreover, although different heating ramps and temperature were assayed during the pyrolysis and atomization steps, which allowed the gradual elimination of the organic matrix and prevented the loss of the analyte, it was not possible to overcome the interference caused by Fe. Therefore, the need of a back-extraction procedure to separate the analyte from the MIL extraction phase before its determination by ETAAS was established in this work. In order to perform the evaluation of the extraction conditions for Cd with the proposed MIL-DLLME method, 500 μL of a 1% (*v*/*v*) HNO_3 solution was used for back-extraction and 20 μL of the this solution were injected into the graphite furnace. This volume and concentration of the back-extraction agent was going to be confirmed later by proper optimization studies.

The optimization of the graphite furnace program was performed in the back-extraction phase. The influence of the pyrolysis temperature and atomization on the absorbance was studied in the range of 300–700 and 1200–1600 °C, respectively. The optimum pyrolysis temperature was 500 °C, higher temperatures caused a significant loss of the analyte, and it was observed that the analytical signal decreased. Once the pyrolysis conditions were selected, the effect of the atomization temperature was evaluated. Optimal atomization time and temperature were found to be 4 s and 1400 °C, respectively. To reduce potential matrix interferences and increase accuracy, the use of different matrix modifiers and mix of modifiers was studied. Individual amounts of each modifier (5 μg Pd, 3 μg Mg, and 50 μg $NH_4H_2PO_4$) and their mixtures (5 μg Pd + 3 μg Mg and 3 μg Mg + 50 μg $NH_4H_2PO_4$) were tested. These modifiers were chosen for this study as they typically provide good thermal stabilization of Cd in the graphite furnace [19]. Moreover, the mixture composed of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ has been frequently used as a matrix modifier for Cd determination in honey, while the mixture composed of $Pd(NO_3)_2$ and $Mg(NO_3)_2$—considered a universal modifier—it has not been widely explored for the analysis of this type of samples [4]. Based on the experiments performed in this work, the best signal-to-background ratio for Cd determination by ETAAS was achieved with 3 μg of Mg + 50 μg $NH_4H_2PO_4$ and other conditions as described in Table 1.

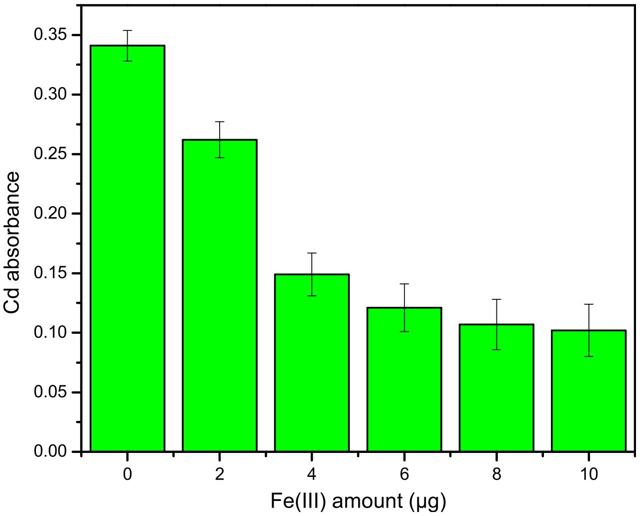

Fig. 1 Effect of different amounts of Fe(III) on Cd signal. A volume of 20 μL of a 2-μg L^{-1} Cd aqueous standard solution was injected. ETAAS instrumental conditions are as indicated in Table 1

Studies on complex formation

The extraction of elements by hydrophobic ILs normally proceeds via the formation of a low polar complex formed with an organic chelating reagent that makes more efficient their extraction into these media [20]. Therefore, the complexation of Cd with an appropriate reagent was studied in this work. Since Cd^{2+} species can occur under this chemical form until pH 8, but Fe^{3+} in the MIL phase is only stable until pH 2 [21], it was anticipated the need of forming a complex with Cd(II) at pH below 2 in order to avoid a possible chemical decomposition of the MIL. Moreover, it is well-known that Cd(II) forms a stable complex with DDTP at pH values below 3 [3], which made this reagent an excellent choice for the formation of a hydrophobic chelate in very acidic conditions. Other typical organic reagents such as diethyl dithiocarbamate (DDTC) and ammonium pyrrolidine dithiocarbamate (APDC) that have been widely applied for the extraction of elements in organic solvents were discarded due to the lack of stability of their complexes with Cd(II) at pHs below 2. Finally, the DDTP reagent was chosen in this work to improve the affinity of Cd for the MIL phase.

The effect of pH on the formation of the Cd-DDTP complex and extraction efficiency was studied in the range of 0.2 to 2, which was adjusted with HCl. The MIL used for this study was $[P_{6,6,6,14}]FeCl_4$. An extraction efficiency of about 70% was obtained for pHs within 0.2 to 0.5, followed by a marked decreased in the pH range of 0.5–2 (see Electronic Supplementary Material (ESM) Fig. S1a). Therefore, 0.5 was selected as optimal pH for next experiments. Subsequently, the effect of DDTP concentration was studied, since it was important to establish a minimum concentration of reagent to guarantee both the total formation of the complex and the highest extraction efficiency. Different molar ratios of Cd/DDTP from 1:0 to 1:1000 were evaluated. The extraction of Cd was not efficient in the absence of DDTP, thus confirming that the reagent was necessary to form a low polar metal complex and achieve optimal extraction efficiency. Likewise, it was observed that a molar ratio of 1:10 was optimal to reach the highest extraction efficiency. For this reason, a 1:10-M ratio was selected for subsequent experiments.

Selection of MIL as extraction solvent

Imidazolium and phosphonium family MILs were evaluated as extraction agents during the development of the proposed DLLME method due to their high hydrophobicity and the possibility of avoiding the centrifugation stage by simple application of a magnetic field provided by an external magnet. In this work, the MIL phase was separated from the aqueous solution using a neodymium magnet (50 (L) × 30 (W) × 10 mm (H)) with a magnetic field induction of 1.17 T and a magnetic susceptibility of 5.89 emu K mol^{-1} [17].

The $[C_8mim]FeCl_4$ MIL was tested as extraction agent in the proposed work; however, it showed a remarkable degree of solubility with the aqueous phase and unsatisfactory extraction percentages were obtained due to significant loss of the extractant phase. Moreover, the use of $[P_{6,6,6,14}]FeCl_4$ MIL was studied. This MIL formed a biphasic system as a consequence of its immiscibility with water, which led to higher Cd extraction than that achieved with $[C_8mim]FeCl_4$ (36%). Furthermore, a MIL formed with the $[P_{6,6,6,14}]^+$ cation and $FeCl_4^{2-}$ anion was studied in this work as Fe(II) can be more stable than Fe(III) within a wider pH range [21]. However, $[P_{6,6,6,14}]_2FeCl_4$ was poorly attracted by the magnet and, hence, the separation of the extraction phase was more difficult than in the case of $[P_{6,6,6,14}]FeCl_4$. This phenomenon can be explained by the weaker magnetic susceptibility of $[P_{6,6,6,14}]_2FeCl_4$ compared with $[P_{6,6,6,14}]FeCl_4$, caused by the high spin of Fe in the $FeCl_4^{-2}$ complex, with four unpaired electrons in its outermost d orbital, while five unpaired electrons occur in $FeCl_4^-$. Thus, Fe(III) is a more paramagnetic species than Fe(II) [22]. Since the separation of $[P_{6,6,6,14}]_2FeCl_4$ phase was difficult by a magnet, centrifugation was applied to completely separate the MIL phase. However, a relatively low extraction efficiency (48%) was obtained with $[P_{6,6,6,14}]FeCl_4$. Therefore, $[P_{6,6,6,14}]FeCl_4$ was selected as extraction phase to develop the MIL-DLLME method.

Determination of the amount of MIL and sample volume

Due to its high hydrophobicity and immiscibility with water, $[P_{6,6,6,14}]FeCl_4$ was considered as a good solvent to form biphasic systems [14]. However, the minimal amount of $[P_{6,6,6,14}]FeCl_4$ leading to the highest extraction efficiency was critical to be established. Likewise, a high sample-to-extraction phase ratio had to be obtained in order to achieve the highest preconcentration factor possible, while an optimal analyte recovery also could depend on MIL amount. Different volumes (20–120 μL) of $[P_{6,6,6,14}]FeCl_4$ extractant phase (50% (*w*/*v*) of MIL prepared in chloroform) were assayed in this work and the results are shown in Fig. S2a in the ESM. The extraction efficiency of the proposed system increased significantly with the increase of MIL volume. A volume of 100 μL of the MIL was enough to reach the highest extraction efficiency obtained at this point of the study. No significant changes in the extraction efficiency were observed when higher volumes of MIL were added to the system. According to this, 100 μL of $[P_{6,6,6,14}]FeCl_4$ extractant phase was chosen for the following experiments.

The effect of the sample volume on Cd extraction was also studied because higher preconcentration factors can be obtained at higher sample-to-extraction phase volume ratios. In this work, the experiments where performed with different volumes of a 0.05-μg Cd L^{-1} standard solution. Since the beginning, it was expected that the higher the sample volume, the more affected could be the extraction efficiency due to the greater solubilization of the MIL in the aqueous phase. However, the extraction efficiency was not significantly diminished for sample volumes up to 30 mL because the MIL phase remained immiscible with the aqueous phase. As shown in Fig. S2b (see the ESM), the highest extraction efficiency was obtained for 5 and 10 mL of sample; nevertheless, these volumes yielded poor preconcentration factors due to a lower sample-to-extractant phase volume ratio than that obtained with 30 mL. Therefore, a volume of 30 mL of sample was selected as optimal, since it showed the best compromise between extraction efficiency and preconcentration factor.

Optimization of the dispersion conditions

A variety of solvents including methanol, chloroform, and acetonitrile were evaluated as dispersants in this work. The effect of 100–500 μL of solvents on Cd extraction was assayed. As can be seen in Fig. 2, the extraction efficiency was increased using acetonitrile up to 300 μL and then it was slightly reduced for higher volumes, probably due to higher solubilization of the MIL phase in the aqueous solution. Finally, 300 μL of acetonitrile were chosen as the optimum volume to obtain a correct dispersion.

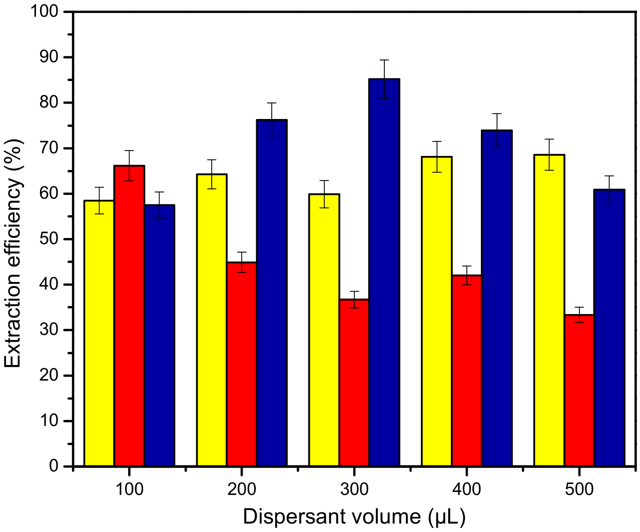

Fig. 2 Effect of type and volume of dispersant on Cd extraction performance of the MIL-DLLME method. Yellow, methanol; red, chloroform; and blue, acetonitrile. Extraction conditions are as shown in Table 2

The efficient mixing of the extraction phase and sample solution is usually one of the most time-consuming steps in extraction methods. For this reason, it is important to set the minimum stirring time to achieve both the highest extraction efficiency and the highest analytical frequency. Thus, the effect of the type and time of stirring on Cd extraction was evaluated using vortex stirring or ultrasound from 0 to 25 min. Among these two approaches, vortex stirring was found to be more effective (Fig. 3). Extraction efficiency close to 50% was achieved with only 1 min of vortex agitation, meaning that the distribution of the analyte between the phases occurs very quickly. The extraction efficiency was increased up to 89% within 15 min and afterwards it was progressively decreased. This effect might be explained considering a partial decomposition of the Cd-DDTP complex for long stirring time, which could favor the transference of free Cd form the MIL towards the aqueous phase. Based on these results, vortex stirring during 15 min was selected for further studies.

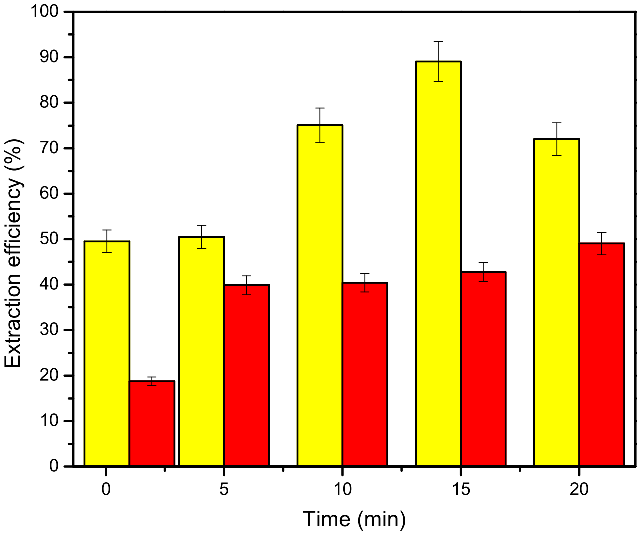

Fig. 3 Effect of type and time of agitation on Cd extraction. Yellow, vortex; red, ultrasound. Other conditions are as mentioned in Tables 1 and 2

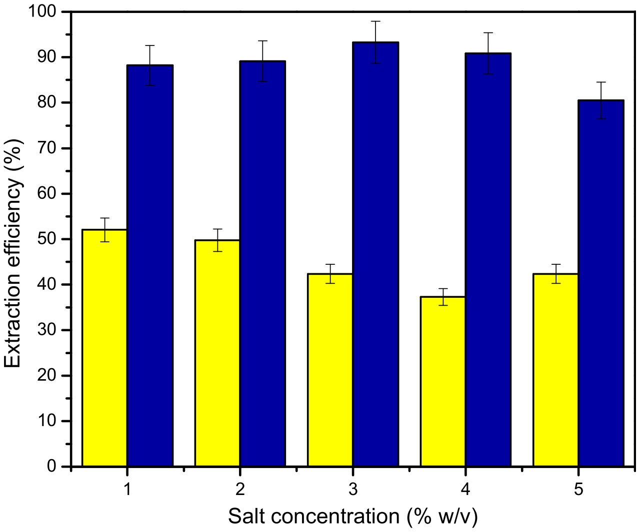

Fig. 4 Influence of the ionic strength on Cd extraction. Yellow, $NaNO_3$; blue, NaCl. Other conditions are as mentioned in Tables 1 and 2

Influence of ionic strength

The addition of salt in LLE procedures can increase the extraction performance due to salting out effect. This effect was studied in this work using NaCl and $NaNO_3$ in a concentration range of 0–5% (*w*/*v*). The extraction efficiency was slightly increased from 89 to 93% when NaCl concentration was increased up to 3% and remained constant up to 5% (*w*/*v*) (Fig. 4). On the other hand, $NaNO_3$ showed lower extraction efficiency in comparison with NaCl at any concentration assayed in this work. This can be explained considering than Cl^- is earlier than NO_3^- in the Hofmeister series, i.e., Cl^- favors more efficiently the hydrophobic interaction of Cd-DDTP complex with the MIL phase, yielding higher extraction efficiency than that obtained in the presence of NO_3^-. Finally, a 3% (*w*/*v*) NaCl was selected for subsequent experiments.

Conditions for back-extraction of Cd from the MIL phase

As mentioned earlier in the "Preliminary studies on Cd determination by ETAAS in the MIL matrix" section, the direct determination of Cd in the enriched MIL phase was not feasible due to the strong interference effect caused by Fe occurring in this type of solvent. Therefore, back-extraction was proposed to fully recover the analyte from the MIL phase after

Table 2 Preconcentration conditions for Cd determination in honey

Extraction conditions	
Sample solution volume	30 mL (1% (*w*/*v*) honey in 1% (*v*/*v*) HNO_3)
Working pH	0.5
DDTP concentration	2×10^{-6} mol L^{-1}
Salt concentration	3% (*w*/*v*) NaCl
MIL volume	100 μL $[P_{6,6,6,14}]FeCl_4$
Dispersant volume	300 μL acetonitrile
Stirring type and time	Vortex, 15 min
Back-extraction conditions	
Back-extraction agent	500 μL of 1% (*v*/*v*) HNO_3
Stirring type and time	Ultrasound, 10 min
Sample injection	20 μL

extraction. Different concentrations and volumes of H_2O_2, HNO_3, HCl, and H_2SO_4 with simultaneous application of ultrasound during 5 to 30 min were assayed for the back-extraction of Cd. In this particular study, solutions with concentrations of these reagents in the range of 1 to 5% (*v*/*v*) and volumes between 100 and 500 μL were tested. The highest Cd back-extraction efficiency was obtained using 500 μL of 1% (*v*/*v*) HNO_3. These conditions were also compatible with the determination of Cd by ETAAS, as diluted HNO_3 is preferred for reproducible and accurate results. Furthermore, it has to be mentioned that when the volumes of HNO_3 acid were lower than 100 μL, Cd was not fully recovered from the MIL phase, most probably due to insufficient mixing of the phases even with the simultaneous application of ultrasound. A volume of 500 μL of 1% (*v*/*v*) HNO_3 and ultrasound for 10 min were the conditions used for back-extraction (Table 2).

Potential interfering species

Several cations such as Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, and Zn^{2+}; toxic elements including Al^{3+}, Pb^{2+}, and Hg^{2+}; and common anions such as Cl^{-}, NO_3^{-}, SO_4^{2-}, and PO_4^{3-} are regularly found in honey samples [23]. For this reason, its influence on the determination of Cd by the proposed method was evaluated. The study was carried out by analyzing a standard solution at 0.05 μg Cd L^{-1} containing each concomitant ion at concentration levels in which they may be commonly present in the samples. In this case, it was considered that a concomitant ion interfered when it produced a variation of the analytical signal by ± 5%. The results showed that the analyzed ions did not generate interference effects up to 300 μg L^{-1} in the case of toxic elements and up to 1000 mg L^{-1} for essential cations and common anions. The ions generally present in the samples under study did not produce any significant interference in the range under study.

Furthermore, it was considered a possible effect of typical organic compounds occurring in honey matrix on Cd extraction and preconcentration. Honey contains carbohydrates as the main components, which represent around 75% of its total mass [23]. Moreover, since glucose and fructose can be found as main components, an interference study was carried out with these carbohydrates at concentrations commonly found in honey. No significant interference effects were observed in the range studied indicating that both major and minor components of honey did not deteriorate the Cd absorbance signal under the conditions mentioned in Table 2.

Analytical performance

The calibration graph obtained after the application of the proposed MIL-DLLME method for the determination of Cd in honey samples was linear from levels near the limit of detection (LOD) and up to at least 250 ng L^{-1}, with a correlation coefficient of 0.9983. The LOD was 0.4 ng L^{-1} Cd (0.04 μg Cd kg^{-1} of honey), calculated as the signal at the intersection plus threefold the standard deviation on the regression of the calibration curve [24]. The relative standard deviation (RSD) was 3.8%, calculated based on the measurement of ten replicates at 0.05 μg L^{-1} Cd. Likewise, possible matrix effects were evaluated in this work. The slopes of different calibration graphs obtained for honey samples diluted at 1, 5, and 10% (*w*/*v*) (at 1% (*v*/*v*) HNO_3) and spiked at different concentrations of Cd were compared with the calibration graph of aqueous standards. It was observed that only when the honey samples were diluted at 1% (*w*/*v*) that the slope of the calibration graph was not different from that obtained with

Table 3 Analytical methods reported in the literature for Cd preconcentration by DLLME

Extractant (volume)	Chelating reagent	Dispersant (volume)	Detection	LOD (ng L^{-1})	RSD (%)	EF	Linear range (ng L^{-1})	Sample	Ref.
$[C_6mim]PF_6$ (73 μL)	DDTC	Water (10 mL)	ETAAS	7.4	3.3	67	20–150	Water	[27]
CCl_4 (35 μL)	DDTP	Acetonitrile (300 μL)	FAAS	20	< 20	–	3000–10,000	Honey	[9]
CCl_4 (140 μL)	DDTP	Methanol (500 μL)	ETAAS	5.6	4.5	116	20–1000	Human teeth	[28]
CCl_4 (34 μL)	APDC	Methanol (500 μL)	ETAAS	0.6	3.5	125	2–20	Water	[29]
$[P_{6,6,6,14}]FeCl_4$ (100 μL)	DDTP	Acetonitrile (300 μL)	ETAAS	0.4	3.8	112	2–250	Honey	Proposed method

Table 4 Results of Cd determination in honey and recovery study (95% confidence interval; $n = 6$)

Honey sample	Added (ng L^{-1})	Found (ng L^{-1})	Recovery (%)[a]
1	0	163.6 ± 5.3 (16.4)[b]	–
	10	173.4 ± 5.4	98.0
	20	182.7 ± 5.7	95.5
	50	212.8 ± 4.7	98.4
2	0	107.1 ± 4.8 (10.7)[b]	–
	10	117.0 ± 4.7	99.0
	20	126.4 ± 5.1	96.5
	50	156.2 ± 4.1	98.2
3	0	129.4 ± 5.0 (12.5)[b]	–
	10	139.1 ± 5.5	97.0
	20	148.5 ± 5.7	95.5
	50	179.9 ± 4.9	101
4	0	53.4 ± 4.1 (5.3)[b]	–
	10	63.6 ± 4.0	102
	20	72.8 ± 4.6	96.9
	50	102.3 ± 3.7	97.8
5	0	92.1 ± 4.5 (9.2)[b]	–
	10	101.7 ± 4.9	96.2
	20	112.5 ± 5.2	102
	50	141.5 ± 4.3	98.8
6	0	74.5 ± 4.4 (7.4)[b]	–
	10	84.3 ± 4.5	97.7
	20	93.7 ± 4.6	95.9
	50	125.0 ± 3.8	101

[a] (Found − Base)/Added × 100

[b] Concentration of Cd expressed as nanogram Cd per gram of honey

aqueous standards, meaning that matrix effect did not occur for that condition.

The sensitivity enhancement factor (EF) was obtained from the slope ratio of the calibration graphs before and after the application of the MIL-DLLME procedure [25]. The EF obtained with a sample volume of 30 mL was 112. The extraction efficiency was defined as the percentage of total Cd in the aqueous phase extracted into the MIL phase [26]. An extraction efficiency of 93% was obtained when the procedure was developed under optimal experimental conditions (Table 2). The preconcentration procedure (complexation of Cd with DDTP, extraction into the dispersed MIL phase, separation of the MIL phase from the aqueous phase by a magnet, and back-extraction of the analyte) could take approximately 35 min per sample. However, it is possible to treat many samples simultaneously if several magnets are used. Therefore, the frequency of analysis is mainly determined by the speed of ETAAS measurements rather than the time elapsed for extraction. Thus, at least 10 samples per hour can be analyzed with the MIL-DLLME method.

A comparative study on the analytical performance of the proposed method with respect to other works already reported in the literature can be found in Table 3. The proposed method shows a LOD and EF that are comparable or even better than other DLLME methods on Cd determination in honey based on the application of ILs or conventional organic solvents. Additionally, the proposed method presents a wide linear range compared to other methods. Furthermore, the use of the intrinsic magnetism of MILs simplifies the DLLME method because the typical time-consuming centrifugation step is avoided. Moreover, the back-extraction stage allowed the ultra-trace determination of Cd in complex matrices such as MIL extraction phase.

Accuracy study and determination of Cd in honey samples

The proposed MIL-DLLME-ETAAS method was applied for the determination of Cd in several types of honey samples after evaluating its accuracy. In this last case, it has to be mentioned that two candidates for certified reference material (CRM) for trace elements in honey have been previously tested [30]. However, a CRM is still not commercialized, most probably due to problems with the homogeneity of honey.

For this reason, a recovery study at three concentration levels was proposed as an alternative strategy to demonstrate the accurate determination of Cd in the presence of honey matrix. Recovery values were found in the range of 95.5–102% (Table 4) which indicated that the MIL-DLLME method can be suitable for Cd determination in the samples under study. The concentrations found in the honey samples were in the range of 5.3–16.4 ng Cd g^{-1}. These concentration values were below the maximum permissible limits established by MERCOSUR regulation and keep close similarities with those reported in other works on Cd determination in honey [23, 31].

Conclusions

In this work, it has been demonstrated that the magnetic $[P_{6,6,6,14}]FeCl_4$ IL can be an excellent extraction phase, along with the DDTP reagent, for highly efficient extraction and determination of Cd at ultra-trace levels in complex samples such as honey. However, the application of MILs for inorganic preconcentration must be studied carefully, mainly because the high content of Fe or other paramagnetic elements included in their composition may cause strong interference effects during elemental determination by atomic spectrometry.

The application of the MIL has as main advantage the possibility of separating the phases by means of a magnet, which simplifies the DLLME procedure by avoiding centrifugation steps. In addition, the application of a back-extraction step allowed the recovery of the MIL phase, which could be considered as a profitable approach due to its possible reuse. Finally, a very low LOD was obtained in the order of nanogram per liter and acceptable analytical recovery was achieved considering the high complexity of the matrix analyzed. These characteristics make that the MIL-DLLME method can be considered as a valuable tool for ultra-trace Cd determination in honey and an attractive choice for routine laboratories dedicated to food quality control.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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