Journal of Molecular Liquids

Sustainable cadmium extraction from sewage sludge samples: a novel approach with hydrophobic deep eutectic solvents and ultrasound-assisted extraction (HDES-UAE) prior to ICP-MS analysis

--Manuscript Draft--

Manuscript Number:	MOLLIQ-D-23-07717R1
Article Type:	Full length article
Section/Category:	Simple organic liquids and mixtures
Keywords:	Eutectic system; green analytical chemistry; ICP-MS; Sample preparation
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	Paulo Clairmont Lima Gomes, Professor
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	Douglas Faza Franco, Professor
	Jose O. Fernandes, Professor
	Sara C. Cunha, Professor
	Mario H. Gonzalez, Professor
Abstract:	Hydrophobic deep eutectic solvents (HDES) are a subclass of DES, formed by hydrogen bond donor and acceptor species, which have low solubility in hydrophilic systems as their main characteristic. In this work, two different mixtures based on DL-menthol (HDES 1: DL-menthol and acetic acid, 1:1 molar ratio; HDES 2: DL-menthol, acetic acid, and pyruvic acid, 1:1:1 molar ratio) were prepared and their physicochemical properties (density and viscosity) were determined. The materials were characterized using FTIR, TG, DSC, and cadmium determination were made by ICP-MS using the KED mode. The greenness of the methods for preparing the mixtures was assessed using the analytical Eco-Scale metric, with high scores obtained for both procedures. The parameters sample mass, HDES volume, and ultrasonication time were optimized using a 33 Box-Behnken experimental design and the solvents were tested in the extraction of cadmium from a sewage sludge certified reference material, obtaining recoveries of 101 and 104% using HDES 1 and HDES 2, respectively. The LOD and LOQ values were 0.271 and 0.905 µg L-1 (HDES 1), and 0.280 and 0.933 µg L-1 (HDES 2), respectively. The two mixtures provided similar results when applied in the extraction of cadmium from three different sewage samples. For sewage sludge A, the cadmium values obtained were 2.073 ± 0.10 µg g-1 (HDES 1) and 2.103 ± 0.11 µg g-1 (HDES 2). For sewage sludge B, the cadmium values were 2.707 ± 0.20 µg g-1 (HDES 1) and 2.067 ± 0.23 µg g-1 (HDES 2). For a poultry sewage sludge sample, the cadmium values were 6.023 ± 0.84 µg g-1 (HDES 1) and 5.319 ± 0.14 µg g-1 (HDES 2). The sample extraction process was evaluated using AGREE software, obtaining a score of 0.7 (out of a possible maximum score of 1.0). Use of the prepared solvents and extraction using an ultrasound-assisted method

	proved to be a good and sustainable strategy for the extraction of Cd from sewage sludge.
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	Mirian Santos mirian.cristina@unesp.br
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Response to Reviewers:	

UNIVERSIDADE ESTADUAL PAULISTA
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IBILCE / UNESP - CÂMPUS DE SÃO JOSÉ DO RIO PRETO

São José do Rio Preto, January 23rd, 2024

Sub: Revised Manuscript to Journal of Molecular Liquids

Dear Editor in Chief

Ref. No.: Manuscript Number: MOLLIQ-D-23-07717

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We have appreciated the very kind attention with the above manuscript and all suggestions assigned by the reviewer were taken into consideration. The answers to the points raised by the reviewer are presented below. We emphasize that all corrections/insertions made to the manuscript were marked in yellow.

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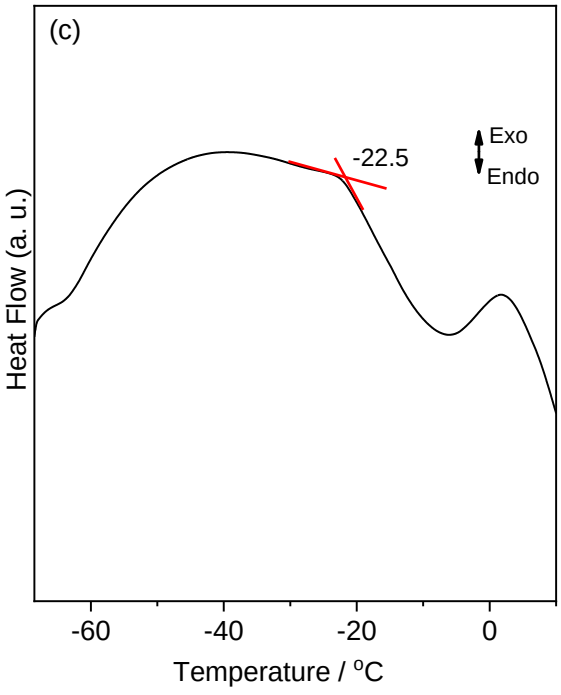

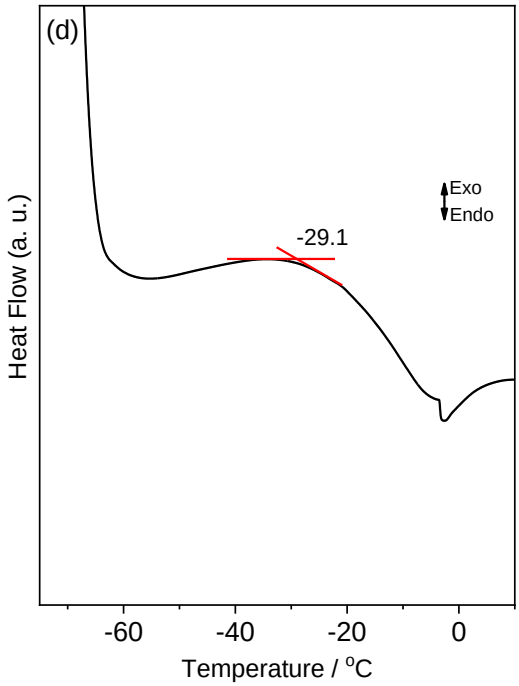

Figure 2: (c-d) DSC curves of the HDES1 and HDES2 solvents (y-axis scale).

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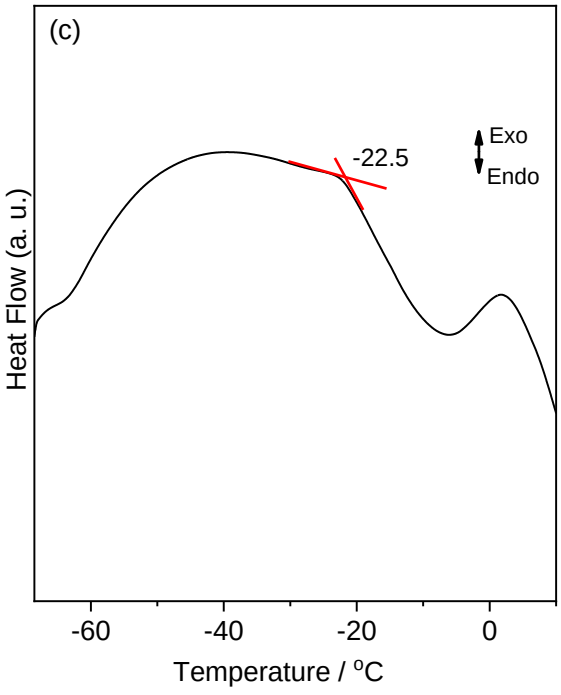


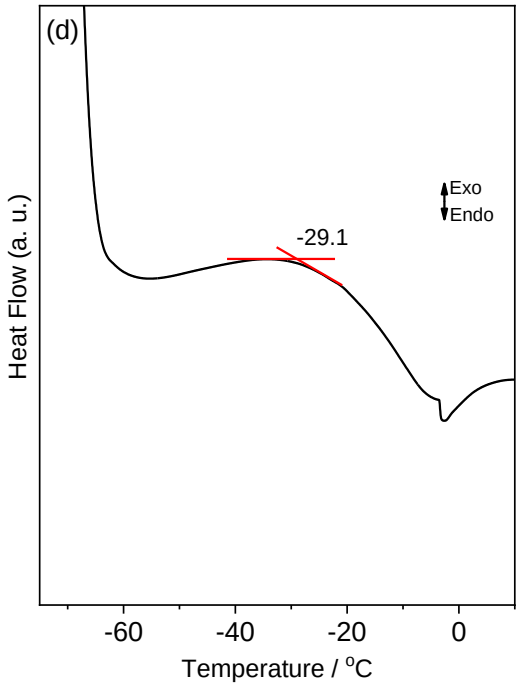
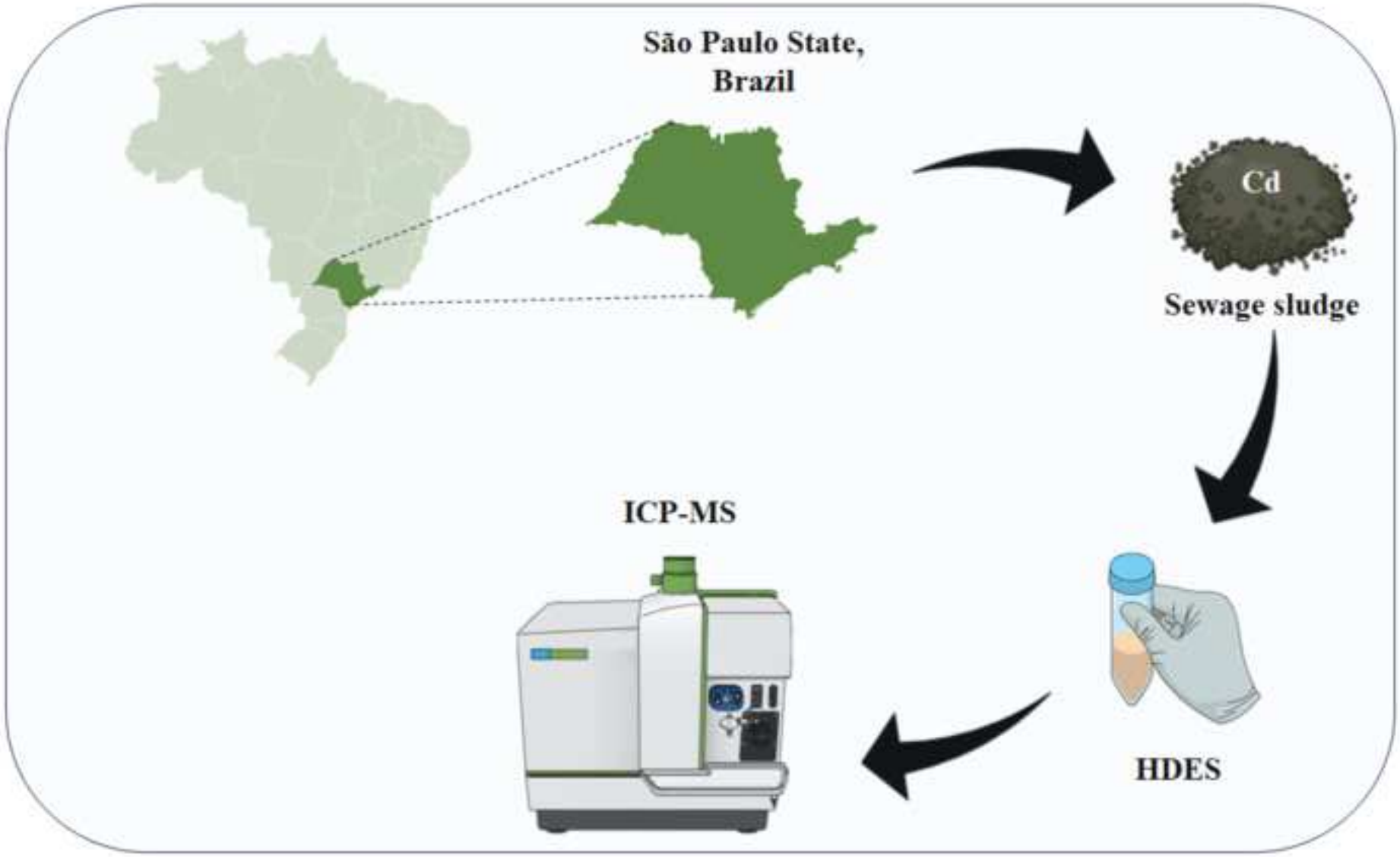


Figure 2: (c-d) DSC curves of the HDES1 and HDES2 solvents (y-axis scale).

Highlights

- Two hydrophobic deep eutectic solvents (HDES) were prepared by stirring and heating and used in the sample preparation step,
- HDES were used for Cadmium extraction from sewage sludge samples,
- The determination of cadmium in the samples was made by ICP-MS,
- Greenness evaluation of eutectic solvent preparation methods

Graphical Abstract



Sustainable cadmium extraction from sewage sludge samples: a novel approach with hydrophobic deep eutectic solvents and ultrasound-assisted extraction (HDES-UAE) prior to ICP-MS analysis

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Abstract

Hydrophobic deep eutectic solvents (HDES) are a subclass of DES, formed by hydrogen bond donor and acceptor species, which have low solubility in hydrophilic systems as their main characteristic. In this work, two different mixtures based on DL-menthol (HDES 1: DL-menthol and acetic acid, 1:1 molar ratio; HDES 2: DL-menthol, acetic acid, and pyruvic acid, 1:1:1 molar ratio) were prepared and their physicochemical properties (density and viscosity) were determined. The materials were characterized using FTIR, TG, DSC, and cadmium determination were made by ICP-MS using the KED mode. The greenness of the methods for preparing the mixtures was assessed using the analytical Eco-Scale metric, with high scores obtained for both procedures. The parameters sample mass, HDES volume, and ultrasonication time were optimized using a 3^3 Box-Behnken experimental design and the solvents were tested in the extraction of cadmium from a sewage sludge certified reference material, obtaining recoveries of 101 and 104% using HDES 1 and HDES 2, respectively. The LOD and LOQ values were 0.271 and 0.905 µg L^{-1} (HDES 1), and 0.280 and 0.933 µg L^{-1} (HDES 2), respectively. The two mixtures provided similar results when applied in the extraction of cadmium from three different sewage samples. For sewage sludge A, the cadmium values obtained were 2.073 ± 0.10 µg g^{-1} (HDES 1) and 2.103 ± 0.11 µg g^{-1} (HDES 2). For sewage sludge B, the cadmium values were 2.707 ± 0.20 µg g^{-1} (HDES 1) and 2.067 ± 0.23 µg g^{-1} (HDES 2). For a poultry sewage sludge sample, the cadmium values were 6.023 ± 0.84 µg g^{-1} (HDES 1) and 5.319 ± 0.14 µg g^{-1} (HDES 2). The sample extraction process was evaluated using AGREE software, obtaining a score of 0.7 (out of a possible maximum score of 1.0). Use of the prepared solvents and extraction using an ultrasound-assisted method proved to be a good and sustainable strategy for the extraction of Cd from sewage sludge.

Keywords: Eutectic system, green analytical chemistry, ICP-MS, sample preparation.

1. Introduction

Deep eutectic solvents (DES), or eutectic mixtures, are a discovery of the last decade with considerable influence in the development of sustainability-based chemical innovations. The use of these solvents has been indicated as a strategy in line with the principles of green analytical chemistry, primarily aiming at reducing the adverse environmental impacts of analytical procedures [1].

DES have the potential to overcome the limitations of ionic liquids, offering advantages such as lower toxicity, higher biodegradability, and simpler preparation [2]. Added to this are the vast range of possible precursor compounds, the ability to modulate the physicochemical properties of these solvents by adjusting the molar ratio or water content, and their excellent capacity to extract organic and inorganic compounds [3]. DES are formed from intermolecular interactions, mainly hydrogen bonds, between two compounds that act as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). This combination can involve the use of liquid or solid components, in pre-established proportions, with the result being a homogeneous liquid mixture [3,4]. Particularly attractive features of DES are that their preparation does not require a solvent or a catalyst, and that no further purification is required before use [4].

The formation of a deep eutectic mixture is characterized by a melting point lower than those of the individual components. Some authors, however, disagree that this condition satisfies the formation of a DES, suggesting that it is necessary for the eutectic point temperature of the solvent to be below that of an ideal liquid mixture, with significant negative deviation from ideality, for it to be considered a deep eutectic solvent [2,5].

DES have been employed in different chemical procedures and in analytical chemistry, and have attracted considerable interest for use in sample pretreatment. The sample preparation procedure is one of the most important steps in an analytical procedure, because the accuracy and precision of the analytical method are largely dependent on this step. The main objectives of the preparation step are sample extraction, analyte purification and enrichment, and possibly modification of the sample to adapt it to the requirements of the analytical apparatus [6,7]. The potential of DES has been evaluated in various extraction and microextraction processes [1]. When using DES for extraction, crucial considerations are the amounts of the raw materials and the length of the alkyl chain, as these can alter the physicochemical properties of the prepared solvents

[8]. However, most of the DES presented in the literature are hydrophilic solvents, which limits their application in aqueous solutions, since their miscibility in aqueous media favors weakening of the intermolecular bonds forming the solvent [3,9].

More recently, this has motivated the development of hydrophobic deep eutectic solvents (HDES), expanding the possible applications and providing even higher efficiencies than hydrophilic DES [10], in addition to being sustainable, low cost, and easy to prepare. These HDES have been successfully applied in water purification [11], preparation of new materials (magnetic gels and nanoparticles based on carbon nanotubes and graphene) for the removal of organic micropollutants and metal ions from water [12-14], CO_2 capture [15], extraction of bioactive compounds [16,17], and the removal of inorganic contaminants, dyes, industrial paints, and pesticides [18]. The HDES are formed by compounds that have low solubility in water, with long-chain fatty acids (such as octanoic, decanoic, or dodecanoic acids) as HBDs, and menthol or N81Cl as HBAs [19].

In the present work, two different mixtures based on DL-menthol were prepared and characterized in terms of their physicochemical properties, prior to use in the sample preparation step in the extraction of cadmium from samples of sewage sludge from a sewage treatment station, as well as from poultry production sewage, employing an ultrasound-assisted extraction (UAE) method and ICP-MS analysis (UAE/ICP-MS). A 3^3 Box-Behnken experimental design was used to maximize the cadmium (Cd) extraction efficiency, evaluating the effects of the variables HDES volume, sample mass, and extraction time. The analytical Eco-Scale was used to assess the greenness of the methods.

2. Experimental procedures

2.1. Materials and reagents

All the materials used were previously decontaminated for 48 h in an acid bath (10% v/v HNO_3), followed by washing with ultrapure deionized water.

The reagents DL-menthol (≥95%) (St. Louis, MO, USA), acetic acid (≥99%) (St. Louis, MO, USA), and pyruvic acid (98%) were purchased from Sigma-Aldrich. A cadmium standard for ICP (1000 ± 2 mg L^{-1}) was purchased from Fluka Analytical (St.

Louis, MO, USA). A sewage sludge certified reference material (CRM) was purchased from SCP Science.

2.2. Preparation and characterization of the HDES

Two different HDES were prepared, using combinations of acetic acid and DL-menthol (1:1 molar ratio), and acetic acid, pyruvic acid, and DL-menthol (1:1:1 molar ratio). The preparation procedure involved stirring for 15 min at 200 rpm, with heating at 60 ºC (AccuPlate Hotplate Stirrer, Model PC-420D, Labnet, Edison, Mexico). Ultrasound treatment employed an ultrasonic bath (Model Tabletop 406 Digital, purchased from SolidSteel) operated at 1500 W and 40 kHz.

Density and viscosity measurements (in triplicate) of the prepared HDES were performed as described by Guimarães et al. [20]. Density was determined using a pycnometer, previously calibrated with ultrapure water, and an analytical balance (Model AG200, Gehaka, Brazil). The viscosity measurements employed a Cannon-Fenske viscometer, calibrated with ultrapure water, at a controlled temperature of 25 ºC.

Infrared spectra were obtained by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), using a Bruker VERTEX 70 instrument operated in the range from 4000 to 400 cm^{-1}, with spectral resolution of 4 cm^{-1} and 64 scans.

Thermogravimetric analyses (TGA) were performed using a TA Instruments SDT Q600 simultaneous TGA/DSC system. The HDES samples were placed in aluminum crucibles and heated from 20 to 200 ºC, at 10.0 ºC min^{-1}, under an atmosphere of nitrogen (N_2) supplied at a flow rate of 10 mL min^{-1}. DSC curves were obtained using a TA Instruments Q20 calorimeter operated at a heating rate of 10 ºC min^{-1}, under an atmosphere of N_2 at a flow rate of 50 mL min^{-1}. Approximately 10 mg portions of the HDES solvents were placed in aluminum crucibles and heated from -70 to 10 ºC. The Q600 software was used to determine the onset and endset temperatures for all thermal events in the TGA and DSC curves.

2.3 Experimental design for cadmium extraction using an ultrasonic bath

The 3^3 Box-Behnken factorial experimental design consisted of three levels (-1, 0, +1), allowing the estimation of first and second order coefficients (linear and quadratic) for the fitted mathematical model, using a minimum number of experiments (15 experiments), with triplicates at the center point. Optimization of the experimental

conditions was performed by the extraction of cadmium from a sewage sludge CRM, using an ultrasonic bath. Experimental designs were implemented separately for HDES 1, which comprised acetic acid and DL-menthol, and HDES 2, which consisted of acetic acid, pyruvic acid, and DL-menthol.

The factors considered in the experimental designs were sample mass (g), volume of HDES (mL), and extraction time (min). The % recovery of cadmium was selected as the response variable, with acceptable values considered to be within the range from 80 to 110% [21]. Table 1 shows the factors, levels, and values used for the experimental design.

Table 1. Factors and values for three levels in the Box-Behnken design.

Factors	**Level -1**	**Level 0**	**Level +1**
CRM sample mass (mg)	150	225	300
Extraction time (min)	15.0	22.5	30.0
HDES volume (mL)	2.0	3.0	4.0

Further information concerning the experimental design is provided in Table S1 (Supplementary Material). Data analysis was performed using Statistica v. 12 software, adopting a confidence level of 95%.

2.4 ICP-MS analysis

Cadmium analysis were carried out employed a NexION 300X ICP-MS instrument (PerkinElmer, Shelton, USA). Conventional nebulization was used, with torch alignment and gas flow set as recommended by the equipment manufacturer. The analyses were carried out using kinetic energy discrimination (KED) mode, with testing of three different He flow rates (2.0, 2.5, and 3.0 mL min^{-1}). Calibration standards were prepared from a 1000 mg L^{-1} cadmium stock solution. For analysis of the extracted sewage, a cadmium calibration curve was constructed with working standards at concentrations in the range from 0.50 to 50 μg L^{-1}, prepared in a matrix-matched medium containing 1.0% HDES 1 (acetic acid and menthol, molar ratio 1:1).

The Box-Behnken design samples were analyzed in both standard mode and using KED, in order to establish the best methodology for the analysis. Table 2 shows all the operating conditions for the determination of cadmium in the samples by ICP-MS.

Table 2. ICP-MS operating conditions.

Parameter	**Operating condition**
Radiofrequency power (kW)	1.6
Plasma gas flow rate (L min^{-1})	18
Auxiliary gas flow rate (L min^{-1})	1.2
Nebulizer gas flow rate (L min^{-1})	1.0
Sample uptake rate (L min^{-1})	0.7
KED mode	
Gas channel	He
He flow rate (mL min^{-1})	2.0 - 3.0
Calibration range (µg L^{-1})	0.50 - 50
Nebulizer	Concentric
Spray chamber	Cyclonic
Isotope (*m/z*)	$^{114}Cd^{+}$
Number of replicates	3

2.5 Quality parameters of the methods for determination of cadmium in the samples

The linearity of the method was assessed by ANOVA (95% confidence level), using triplicate calibration curves in the concentration range 0.500-50.0 µg L^{-1}. For the limits of detection (LOD) and quantification (LOQ), the formulas 3 s/a and 10 s/a were used, where "s" is the standard deviation of ten analytical blanks and "a" is the slope of the analytical curve [21].

The accuracy and precision of the method considered the % recovery and relative standard deviation (RSD %), respectively, obtained using the sewage sludge CRM sample.

2.6 Samples

After determining the optimal extraction condition, the developed methodology was applied to the sewage sludge CRM and to the sludges from two sewage treatment stations (A and B, both in São Paulo state, Brazil), in addition to a sewage sludge from a poultry production facility (also in São Paulo state, Brazil). The sewage sludge samples were collected during the winter of 2023 (in the Southern Hemisphere), in plastic pots that had been previously decontaminated for 24 h in a 10% HNO_3 bath. The samples were dried in an oven at 60 ºC for 72 h, in accordance with EPA guidelines [22]. After drying, the samples were ground and sieved through 63 μm meshes, for subsequent use in the experiments.

3. Results and Discussion

3.1 Density and viscosity measurements

Deep eutectic solvents can be prepared by different methods, such as heating, ultrasound, microwave radiation, lyophilization, or evaporation. In this work, it was decided to use heating, since this method is simple, efficient, and allows the preparation of several DES or HDES simultaneously. Menthol was selected as one of the components, because it is nontoxic, widely available, and an excellent hydrogen bond acceptor [23].

Density and viscosity are important physicochemical properties of HDES, since they directly influence the mass transport phenomena and, consequently, the possible applications of these solvents. Solvents with low viscosity are considered ideal for use in extraction processes, as they allow greater mass transfer. When ICP-MS is used for the analysis of metals, solvents with lower viscosities favor spray formation in the nebulizer, as well as mass transport of the sample to the plasma [24].

The use of precursors rich in hydroxyl groups acts to increase the sites available for the formation of hydrogen bonds, as also observed when using organic acids, which produce solvents that are denser and more viscous. In the present case, it could be seen that the addition of pyruvic acid in the ternary mixture increased the density and viscosity of the solvent, as expected. For HDES 1, the density and viscosity values at 25 ºC were 0.930 ± 0.0051 g mL^{-1} and 5.96 ± 0.026 mPa s, respectively. For HDES 2, the values were 0.988 ± 0.0022 g mL^{-1} and 8.87 ± 0.081 mPa s, respectively.

It is known that for non-ionic HDES, as in the present case, the viscosity increases as the HBD chain increases. This was observed here, since the addition of a second HBD (pyruvic acid) in HDES 2 led to a marked increase in the viscosity of the mixture, when compared to HDES 1. A similar effect was observed in relation to density in work involving the preparation of HDES and NADES [25, 26, 27, 28].

3.2 FTIR analysis

FTIR is widely used to confirm molecular interactions, in addition to being an excellent method of characterization, enabling the confirmation of chemical functional groups and/or molecular bonds. In this way, the formation of a new HDES can be confirmed by the existence of hydrogen bonds between DL-menthol and hydrogen bond donors, which in this case were acetic acid and pyruvic acid. The structures of these hydrogen bond donors result in a characteristic band at approximately ~1700 cm^{-1}, related to the ketone or carboxylic acid groups. The hydrogen bond acceptor (DL-menthol) presents a characteristic band at approximately ~3000 cm^{-1}, related to the hydroxyl group [27]. As shown in Figure 1, the FTIR spectra can confirmed the formation of hydrogen bonds between DL-menthol and the hydrogen bond donors, with the band corresponding to the carboxylic group shifting to higher wavenumbers (~1711 cm^{-1} for the mixture of acetic acid and menthol, and ~1713 cm^{-1} for the mixture of acetic acid, pyruvic acid, and menthol). In addition, the physical state of the HDES formed (liquid and homogeneous for all the mixtures) evidenced the formation of the eutectic mixture, since the reagents were initially in solid form [27].

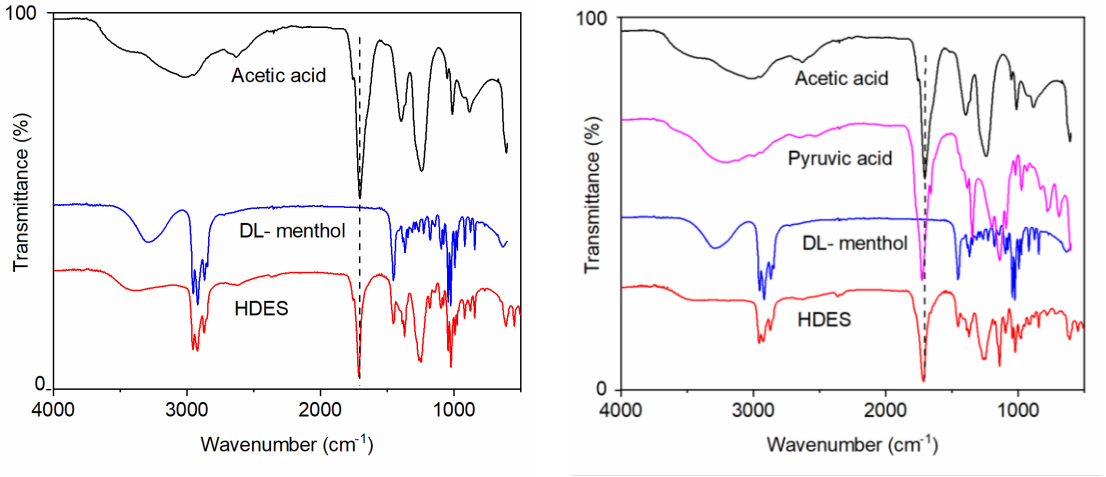

Figure 1. FTIR spectra of HDES 1 (DL-menthol and acetic acid) and HDES 2 (DL-menthol, pyruvic acid, and acetic acid).

3.3 DSC and TG/DTG measurements

The TG/DTG curves for the HDES solvents are shown in Figure 2(a-b). Pyruvic acid was added with the intention of providing greater hydrogen bond-type interactions and consequently increasing interactions between the species of interest. In this paper, for the first time the preparation of HDES from the hydrogen bond donors, acetic acid and pyruvic acid plus the acceptor DL -menthol was reported. The TG curves enabled analysis of the thermal behavior of the two synthesized solvents. It can be observed that the mass loss process for both solvents begins at low temperature values. The TG curves of HDES1 and HDES2 show a significant mass loss up to 155°C and 138°C, respectively. The DTG curves showed the presence of two slopes, with onset points at approximately 41.8 and 97.1 ºC for HDES 1, and at 41.8 and 88.3 ºC for HDES 2. The DTA curves for the solvents (Figure S1, Supplementary Material) showed the presence of two endothermic peaks, with minima at 78 and 136 ºC for HDES 1, and at 56 and 141 ºC for HDES 2. These two endothermic peaks could be attributed to the thermal decomposition of the solvents as the temperature increased.

Figure 2(c-d) displays the DSC curves obtained at low temperatures for the two synthesized eutectic solvents. These curves enabled the determination of the specific temperature at which two or more substances form a single liquid phase, leading to the formation of an ideal liquid mixture [27, 28]. This temperature is recognized as the eutectic point [27, 28]. The eutectic points for the HDES solvents were determined by

tracing two tangent straight lines on the DSC curves. The eutectic point temperatures for HDES 1 and HDES 2 were found to be -22.5 and -29.1 ºC, respectively (Figure 2c-d).

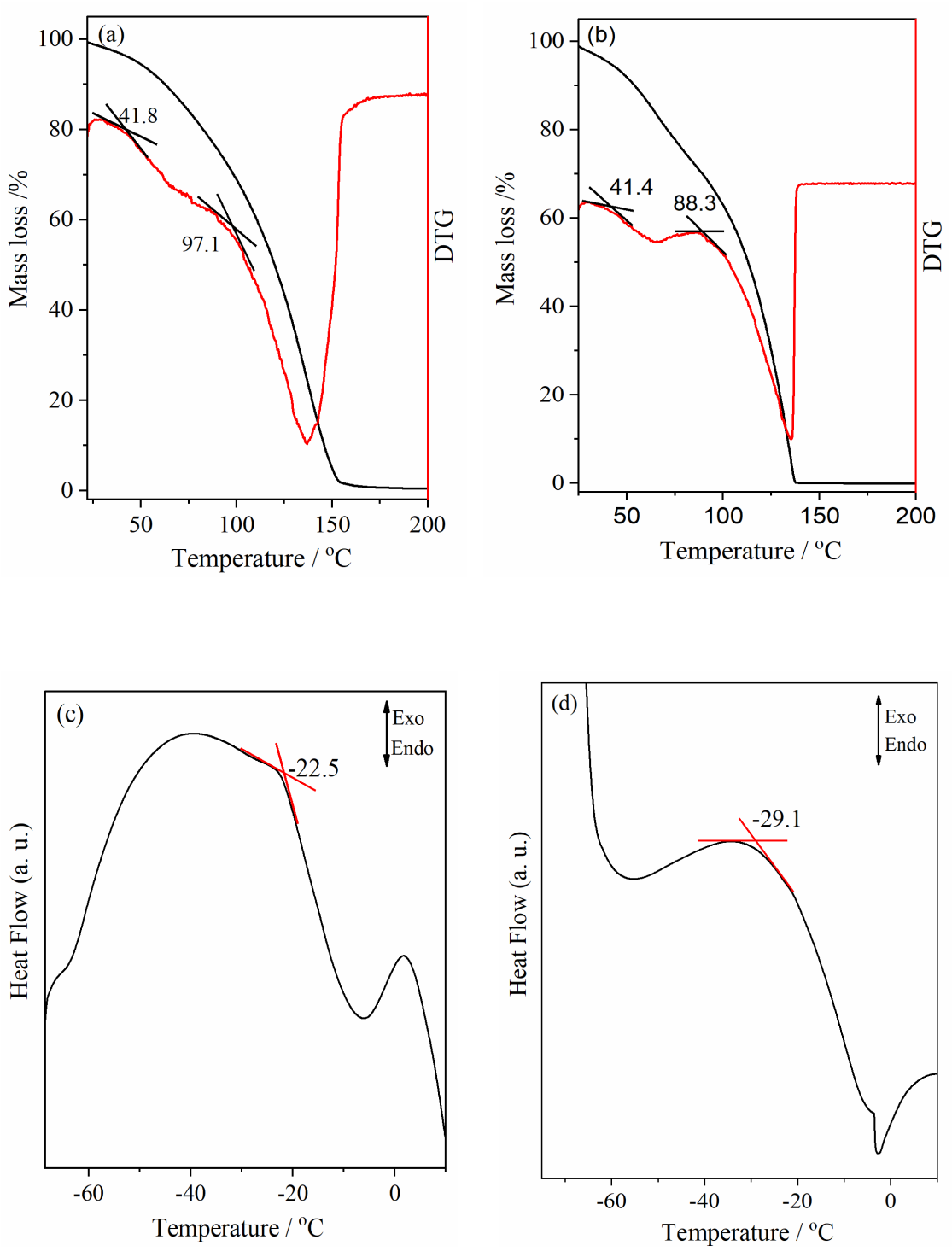

Figure 2. (a-b) TG/DTG and (c-d) DSC curves for HDES 1 and HDES 2.

3.4 Experimental design

The data obtained by ICP-MS revealed that the best analysis condition, based on the recovery results, was in KED mode, with He flow rate of 3.0 mL min^{-1}. This condition was chosen to compose the experimental design and was used at the time of sample analysis.

The results of the experimental design were analyzed separately for HDES 1 and HDES 2, with evaluation based on the recovery percentages obtained using the sewage sludge CRM. Figure 3 presents the results in the form of Pareto charts, adopting a 95% confidence level ($p > 0.05$). For HDES 1, the charts demonstrated the significance of linear (L) and quadratic (Q) variables of the mathematical model. The main effect factors included CRM mass (L), HDES volume (L), and CRM mass (Q) as independent variables, with a residual mean square (RMS) of 19.47. Comparable behavior was observed for HDES 2, where the HDES volume (L), CRM mass (L), and CRM mass (Q) were identified as main effects, with RMS of 22.18. These results indicated satisfactory model fits with first and second order coefficients.

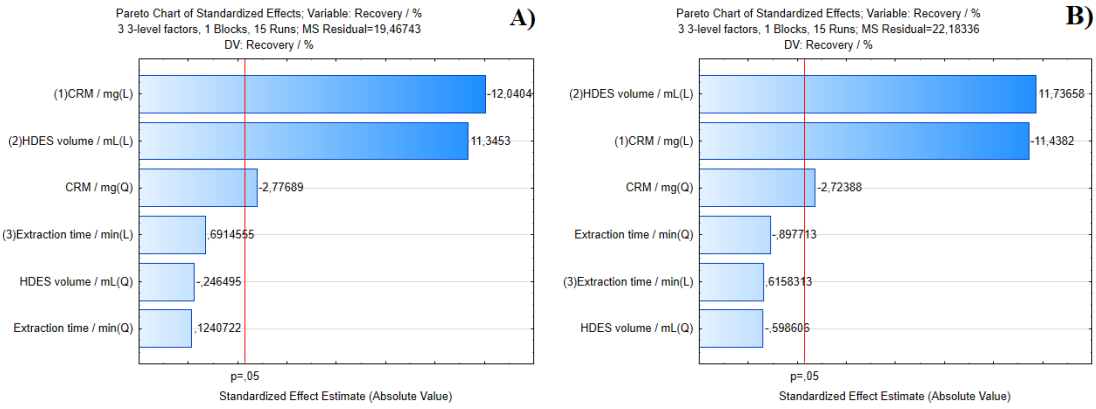

Figure 3. Pareto charts of main effects ($p > 0.05$) for the extraction and recovery of Cd in the CRM using (A) HDES 1 (acetic acid and DL-menthol) and (B) HDES 2 (acetic acid, pyruvic acid, and DL-menthol).

Application of ANOVA (Table S2, Supplementary Material) resulted in correlation coefficients (R^2) of 0.9741 and 0.9719 for HDES 1 and HDES 2, respectively, indicating excellent fits of the mathematical models. The RMS values were 19.467 and 22.183 for HDES 1 and HDES 2, respectively, demonstrating minimal residual variation in the response variable (% recovery) after applying the model. Among the selected

independent variables and their interactions, CRM mass (L) and CRM mass (Q), as well as HDES volume (Q), were identified as the most significant (p = 0.05).

Response surface graphs were plotted for HDES 1 and HDES 2, depicting the relationships between the specified two independent variables (among CRM mass, HDES volume, and extraction time) and the dependent variable (% recovery). For HDES 1, evaluation of the effects of extraction time (min) and CRM mass (mg), shown in Figure 4A, revealed that recoveries above 80% were consistently achieved for extraction times from 15 to 30 min. Notably, the optimal analysis time was 15 min, significantly reducing the ultrasonication time required to achieve satisfactory results. Considering the effect of CRM mass (mg), the best results were obtained with the lowest mass evaluated (150 mg). The surface plot for the effects of extraction time (min) and HDES volume (mL), shown in Figure 4B, revealed that the effect of time was non-significant, with recoveries above 30% obtained for times from 15 to 30 min. However, superior recoveries were observed with larger volumes of extracting solvent, particularly using HDES 1, with volumes exceeding 3.0 mL. For the relationship between CRM mass and HDES volume (Figure 4C), it was observed that lower CRM mass values (ranging from 150 to 200 mg) enabled recoveries above 80%, with HDES volumes exceeding 2.5 mL. These results were associated with satisfactory curvatures of the response surfaces obtained for the use of HDES 1 composed of acetic acid and DL-menthol.

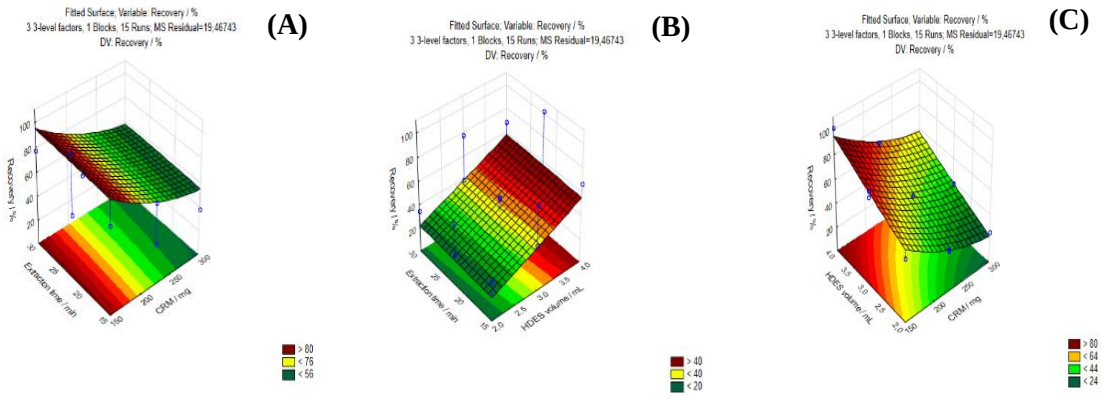

Figure 4. Response surface plots for the effects of the independent variables on % recovery of Cd from the CRM using HDES 1 (acetic acid and DL-menthol): (A) CRM mass (mg) and extraction time (min); (B) extraction time (min) and HDES volume (mL); (C) HDES volume (mL) and CRM mass (mg).

The response surface plots for HDES 2 exhibited behaviors analogous to those observed for HDES 1. The plot obtained with the variables extraction time and CRM mass (Figure 5A) showed that recoveries of 100% were achieved with a smaller CRM mass, ranging between 150 and 180 mg, with time being insignificant. An extraction time of only 15 min was sufficient to achieve satisfactory results. Considering the HDES volume and extraction time (Figure 5B), recoveries exceeding 60% were achieved with larger HDES volumes in the range from 3.0 to 4.0 mL, regardless of the analysis time. Finally, Figure 5C shows that recoveries above 100% could be achieved with CRM mass between 150 and 200 mg and HDES 2 volume between 3.0 and 4.0 mL. Excellent recovery results were observed for HDES 2, with HDES volume between 3.0 and 4.0 mL, regardless of the extraction time, allowing selection of an extraction time of only 15 min in this ultrasound- assisted method. All the response surface graphs presented favorable curvature.

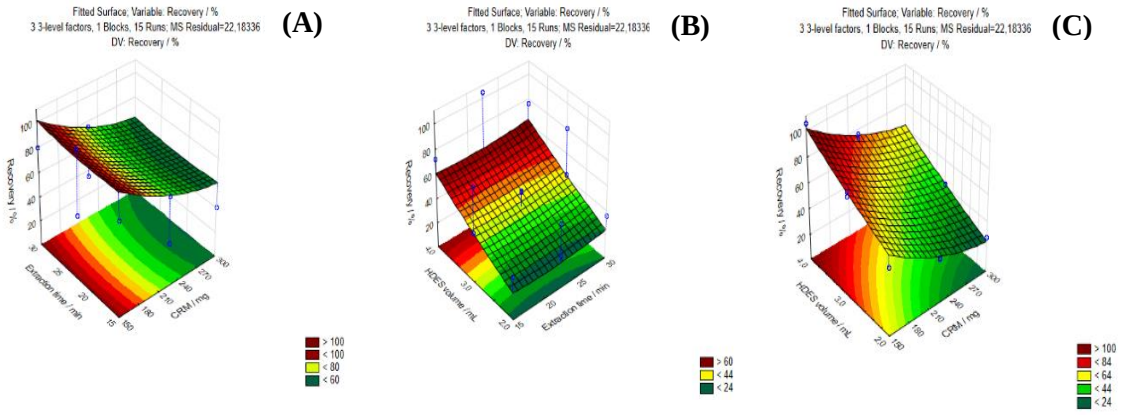

Figure 5. Response surface plots for the effects of the independent variables on % recovery of Cd from the CRM using HDES 2 (acetic acid, pyruvic acid, and DL-menthol): (A) extraction time (min) and CRM mass (mg); (B) HDES volume (mL) and extraction time (min); (C) HDES volume (mL) and CRM mass (mg).

The contour plot data supported the results obtained previously, enabling definition of the optimal conditions for application of the developed methodology. Higher accuracies (101% for HDES 1 and 104% for HDES 2) were obtained using higher HDES volume (4.0 mL) and lower sample mass (150 mg). Therefore, the condition considered optimal was HDES volume of 4.0 mL, sample mass of 150 mg, and extraction time of 15.0 min.

3.5 Quality parameters of the proposed methods

The linearity of the method was evaluated by constructing a calibration curve in the concentration range 0.500-50.0 µg L^{-1}, which could be described by the following equation: y = 1216[Cd] – 939.7; R^2 = 0.999. Application of ANOVA showed no lack of fit for the method (F_{cal} (1.91 x 10^{-12}) < $F_{tab\ 95\%}$ (10.9 x 10^{3})). The LOD and LOQ values were 0.271 and 0.905 µg L^{-1} (HDES 1), and 0.280 and 0.933 µg L^{-1} (HDES 2), respectively (Table 3). The precision (RSD %) of the method was determined as the repeatability of the measurements (n = 3), obtaining values of 2.65% (HDES 1) and 2.34% (HDES 2) (Table 3).

Table 3. Figures of merit for analysis of Cd by UAE/ICP-MS (n = 3) methodology with extraction using HDES 1 and HDES 2.

Analyte	HDES 1			HDES 2		
	LOD (µg L^{-1})	LOQ (µg L^{-1})	Recovery (%)	LOD (µg L^{-1})	LOQ (µg L^{-1})	Recovery (%)
^{114}Cd	0.271	0.905	101	0.280	0.933	104

Cadmium was determined in the sewage sludge and CRM samples using external calibration. The concentrations obtained for sewage sludges A and B and the poultry sewage are summarized in Table 4.

Table 4. Concentrations of Cd in the samples analyzed by the UA/ICP-MS methodology with extraction using HDES 1 and HDES 2.

Analyte	HDES 1				HDES 2			
	Sewage sludge A	Sewage sludge B	Poultry sewage sludge	CRM	Sewage sludge A	Sewage sludge B	Poultry sewage sludge	CRM
^{114}Cd	2.073 ± 0.10[a]	2.707 ± 0.20[a]	6.023 ± 0.84[a]	1.920 ± 0.21[a]	2.103 ± 0.11[a]	2.067 ± 0.23[a]	5.319 ± 0.14[a]	1.981 ± 0.16[a]

[a]Average (µg g^{-1}) ± standard deviation (n = 3).

In the case of these types of samples, it should be noted that environmental variations can cause compositional differences, influenced by factors including climate, season, and anthropic activity. The analytical Eco-Scale method was used to evaluate the HDES preparation procedure. In this technique, a value of 100 is the ideal score, with penalty points being deducted for each parameter that differs from the ideal value (in other words, penalty points are assigned for aspects of methods that do not comply with the principles of green chemistry). The energy consumption for the HDES preparation was calculated, resulting in a value of 0.0392 kWh mL^{-1} for each mixture. The only penalty occurred due to the use of agitation and heating, for which 2 points were deducted. According to Van Aken et al. [29], an Eco-Scale score above 75 represents a green method. Here the HDES preparation method received a score of 98, out of a total of 100 possible points, representing excellent green characteristics [30].

The greenness of the cadmium extraction and determination was evaluated using the AGREE open-source software. This generates a graph with scores that can vary from 0 (lowest greenness) to 1 (highest greenness), based on the 12 principles of green analytical chemistry (GAC) [29]. The graph generated by the software shows a color scale ranging from red to yellow to green, indicating weak aspects of the method (red color) and its strong points (green color). The items described in Figure 6 were evaluated using AGREE, obtaining a final score of 0.7 (without considering the energy consumption of analyzes by ICP-MS), indicating that the developed method could be considered green.

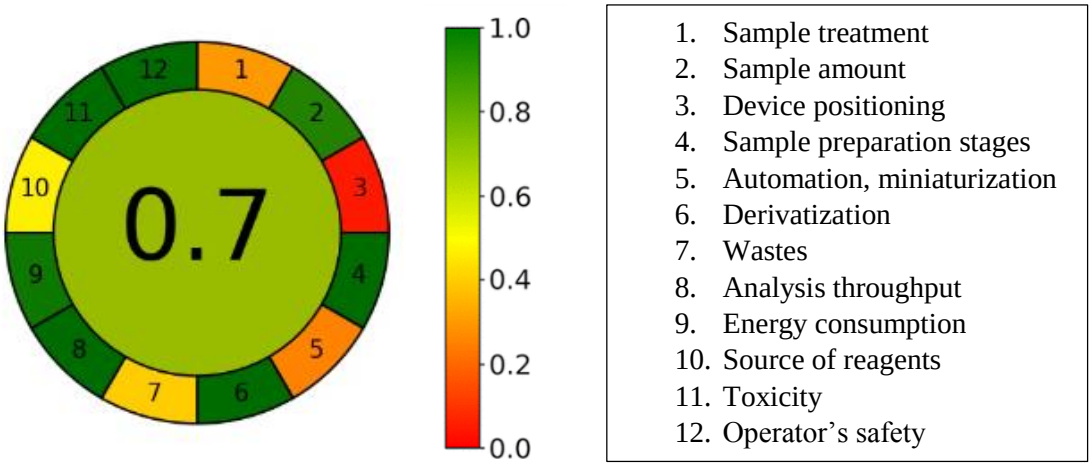

Figure 6. Use of AGREE to evaluate the greenness of the ultrasound-assisted extraction method.

Previous studies have reported the use of HDES prepared from DL-menthol and acetic acid for the extraction of pesticides from aqueous environments [11], the extraction of bisphenol A from aqueous medium [32], and the removal of diclofenac [33], but to date no reports were found concerning the use of this HDES for the removal of cadmium from sewage sludge. The use of HDES prepared using acetic acid, pyruvic acid, and DL-menthol has not previously been reported for any application (including the extraction of cadmium in sewage sludge).

The extraction of cadmium present in sewage sludge is generally performed using acidic solutions (HNO_3), followed by ICP-MS or ICP OES analysis [34,35]. The Table 5 compares the recoveries of cadmium extractions in sewage sludge samples using acidic solutions and the method developed in this paper. The high viscosity of HDES did not cause any impact on the analyses, as it was not used purely at the time of the analyses, it was diluted for use in the ICP (100x dilution) so as not to cause instrumental problems. It should be noted that the use of acidic solutions and microwaves are official methods for preparing samples, although a disadvantage is that they produce toxic residues, in addition to requiring long extraction times that compromise the analytical frequency.

Table 5. Comparison of cadmium extraction efficiency in sewage sludge samples using different solutions

LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	Amount of sample and reagents	Extracting solution viscosity (mPa s)	Recovery (%)	Instrumental apparatus	Refer.
4.60	15.4	0.5 g/ 10 mL HNO_3	0.76	>95	ICP- OES	35
*Nd	*Nd	0.5 g/ 25 mL polyepoxysuccinic acid	*Nd	78	ICP- OES	36
*Nd	*Nd	1g/ 5.0 mL HNO_3 /2.0 mL HCl/ 3.0 mL HF	*Nd	101	ICP-MS	37
0.271	0.905	150 mg/ 4.0 mL HDES (DL-menthol and acetic acid)	5.96	101	ICP-MS	This paper
0.280	0.933	150 mg/ 4.0 mL HDES (DL-menthol, acetic acid, and pyruvic acid)	8.87	104		

* Nd= not described

Conclusions

Two different mixtures containing DL-menthol were prepared by stirring and heating, for subsequent use in the extraction of cadmium from different sewage sludge samples. The mixtures were evaluated using FTIR, DSC, viscosity, and density analyses, and proved to be equally efficient in removing the selected analyte. Box-Behnken experimental design was used for elucidation of the best HDES volume, sample mass, and extraction time to be used in the analysis. The analytical Eco-Scale method was used to evaluate the HDES preparation method, showing that the method could be considered a green technique that complied with the principles of green chemistry. The cadmium values obtained using HDES 1 and HDES 2 with sewage sludge A were 2.073 ± 0.10 µg g^{-1} (HDES 1) and 2.103 ± 0.11 µg g^{-1} (HDES 2). For sewage sludge B, the values obtained were 2.707 ± 0.20 µg g^{-1} (HDES 1) and 2.067 ± 0.23 µg g^{-1} (HDES 2). Finally, the values obtained with the poultry sewage sludge sample were 6.023 ± 0.84 µg g^{-1} (HDES 1) and

5.319 ± 0.14 μg g^{-1} (HDES 2). The sample preparation time was short (15 min), which provided high analytical frequency. Use of the prepared HDES and the ultrasound-assisted extraction method proved to be an excellent option for the extraction of cadmium from different sewage sludges, as a substitute for the nitric acid solution and microwave treatment conventionally employed in the sample preparation step.

Acknowledgments

The authors are grateful for the financial support provided by the São Paulo State Research Foundation (FAPESP, grants #2021/14581-1, #2021/14759-5, #2023/04832-2, #2019/22113-8, #2017/18531-3, and #2014/50945-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant #465571/2014-0 and grant #403839/2023-8), and Instituto Nacional de Tecnologias Alternativas para Detecção, Avaliação Toxicológica e Remoção de Micropoluentes e Radioativos (INCT-DATREM). Sara C. Cunha acknowledges FCT for the 2022.07841.CEECIND/CP1724/CT0014 contract. The LAQV-REQUIMTE authors thanks FCT Fundação para a Ciência e a Tecnologia through national funds to the project CleanH20 2022.03009.PTDC

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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.

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Sustainable cadmium extraction from sewage sludge samples: a novel approach with hydrophobic deep eutectic solvents and ultrasound-assisted extraction (HDES-UAE) prior to ICP-MS analysis

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Abstract

Hydrophobic deep eutectic solvents (HDES) are a subclass of DES, formed by hydrogen bond donor and acceptor species, which have low solubility in hydrophilic systems as their main characteristic. In this work, two different mixtures based on DL-menthol (HDES 1: DL-menthol and acetic acid, 1:1 molar ratio; HDES 2: DL-menthol, acetic acid, and pyruvic acid, 1:1:1 molar ratio) were prepared and their physicochemical properties (density and viscosity) were determined. The materials were characterized using FTIR, TG, DSC, and cadmium determination were made by ICP-MS using the KED mode. The greenness of the methods for preparing the mixtures was assessed using the analytical Eco-Scale metric, with high scores obtained for both procedures. The parameters sample mass, HDES volume, and ultrasonication time were optimized using a 3^3 Box-Behnken experimental design and the solvents were tested in the extraction of cadmium from a sewage sludge certified reference material, obtaining recoveries of 101 and 104% using HDES 1 and HDES 2, respectively. The LOD and LOQ values were 0.271 and 0.905 µg L^{-1} (HDES 1), and 0.280 and 0.933 µg L^{-1} (HDES 2), respectively. The two mixtures provided similar results when applied in the extraction of cadmium from three different sewage samples. For sewage sludge A, the cadmium values obtained were 2.073 ± 0.10 µg g^{-1} (HDES 1) and 2.103 ± 0.11 µg g^{-1} (HDES 2). For sewage sludge B, the cadmium values were 2.707 ± 0.20 µg g^{-1} (HDES 1) and 2.067 ± 0.23 µg g^{-1} (HDES 2). For a poultry sewage sludge sample, the cadmium values were 6.023 ± 0.84 µg g^{-1} (HDES 1) and 5.319 ± 0.14 µg g^{-1} (HDES 2). The sample extraction process was evaluated using AGREE software, obtaining a score of 0.7 (out of a possible maximum score of 1.0). Use of the prepared solvents and extraction using an ultrasound-assisted method proved to be a good and sustainable strategy for the extraction of Cd from sewage sludge.

Keywords: Eutectic system, green analytical chemistry, ICP-MS, sample preparation.

1. Introduction

Deep eutectic solvents (DES), or eutectic mixtures, are a discovery of the last decade with considerable influence in the development of sustainability-based chemical innovations. The use of these solvents has been indicated as a strategy in line with the principles of green analytical chemistry, primarily aiming at reducing the adverse environmental impacts of analytical procedures [1].

DES have the potential to overcome the limitations of ionic liquids, offering advantages such as lower toxicity, higher biodegradability, and simpler preparation [2]. Added to this are the vast range of possible precursor compounds, the ability to modulate the physicochemical properties of these solvents by adjusting the molar ratio or water content, and their excellent capacity to extract organic and inorganic compounds [3]. DES are formed from intermolecular interactions, mainly hydrogen bonds, between two compounds that act as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). This combination can involve the use of liquid or solid components, in pre-established proportions, with the result being a homogeneous liquid mixture [3,4]. Particularly attractive features of DES are that their preparation does not require a solvent or a catalyst, and that no further purification is required before use [4].

The formation of a deep eutectic mixture is characterized by a melting point lower than those of the individual components. Some authors, however, disagree that this condition satisfies the formation of a DES, suggesting that it is necessary for the eutectic point temperature of the solvent to be below that of an ideal liquid mixture, with significant negative deviation from ideality, for it to be considered a deep eutectic solvent [2,5].

DES have been employed in different chemical procedures and in analytical chemistry, and have attracted considerable interest for use in sample pretreatment. The sample preparation procedure is one of the most important steps in an analytical procedure, because the accuracy and precision of the analytical method are largely dependent on this step. The main objectives of the preparation step are sample extraction, analyte purification and enrichment, and possibly modification of the sample to adapt it to the requirements of the analytical apparatus [6,7]. The potential of DES has been evaluated in various extraction and microextraction processes [1]. When using DES for extraction, crucial considerations are the amounts of the raw materials and the length of the alkyl chain, as these can alter the physicochemical properties of the prepared solvents

[8]. However, most of the DES presented in the literature are hydrophilic solvents, which limits their application in aqueous solutions, since their miscibility in aqueous media favors weakening of the intermolecular bonds forming the solvent [3,9].

More recently, this has motivated the development of hydrophobic deep eutectic solvents (HDES), expanding the possible applications and providing even higher efficiencies than hydrophilic DES [10], in addition to being sustainable, low cost, and easy to prepare. These HDES have been successfully applied in water purification [11], preparation of new materials (magnetic gels and nanoparticles based on carbon nanotubes and graphene) for the removal of organic micropollutants and metal ions from water [12-14], CO_2 capture [15], extraction of bioactive compounds [16,17], and the removal of inorganic contaminants, dyes, industrial paints, and pesticides [18]. The HDES are formed by compounds that have low solubility in water, with long-chain fatty acids (such as octanoic, decanoic, or dodecanoic acids) as HBDs, and menthol or N81Cl as HBAs [19].

In the present work, two different mixtures based on DL-menthol were prepared and characterized in terms of their physicochemical properties, prior to use in the sample preparation step in the extraction of cadmium from samples of sewage sludge from a sewage treatment station, as well as from poultry production sewage, employing an ultrasound-assisted extraction (UAE) method and ICP-MS analysis (UAE/ICP-MS). A 3^3 Box-Behnken experimental design was used to maximize the cadmium (Cd) extraction efficiency, evaluating the effects of the variables HDES volume, sample mass, and extraction time. The analytical Eco-Scale was used to assess the greenness of the methods.

2. Experimental procedures

2.1. Materials and reagents

All the materials used were previously decontaminated for 48 h in an acid bath (10% v/v HNO_3), followed by washing with ultrapure deionized water.

The reagents DL-menthol (≥95%) (St. Louis, MO, USA), acetic acid (≥99%) (St. Louis, MO, USA), and pyruvic acid (98%) were purchased from Sigma-Aldrich. A cadmium standard for ICP (1000 ± 2 mg L^{-1}) was purchased from Fluka Analytical (St.

Louis, MO, USA). A sewage sludge certified reference material (CRM) was purchased from SCP Science.

2.2. Preparation and characterization of the HDES

Two different HDES were prepared, using combinations of acetic acid and DL-menthol (1:1 molar ratio), and acetic acid, pyruvic acid, and DL-menthol (1:1:1 molar ratio). The preparation procedure involved stirring for 15 min at 200 rpm, with heating at 60 ºC (AccuPlate Hotplate Stirrer, Model PC-420D, Labnet, Edison, Mexico). Ultrasound treatment employed an ultrasonic bath (Model Tabletop 406 Digital, purchased from SolidSteel) operated at 1500 W and 40 kHz.

Density and viscosity measurements (in triplicate) of the prepared HDES were performed as described by Guimarães et al. [20]. Density was determined using a pycnometer, previously calibrated with ultrapure water, and an analytical balance (Model AG200, Gehaka, Brazil). The viscosity measurements employed a Cannon-Fenske viscometer, calibrated with ultrapure water, at a controlled temperature of 25 ºC.

Infrared spectra were obtained by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), using a Bruker VERTEX 70 instrument operated in the range from 4000 to 400 cm^{-1}, with spectral resolution of 4 cm^{-1} and 64 scans.

Thermogravimetric analyses (TGA) were performed using a TA Instruments SDT Q600 simultaneous TGA/DSC system. The HDES samples were placed in aluminum crucibles and heated from 20 to 200 ºC, at 10.0 ºC min^{-1}, under an atmosphere of nitrogen (N_2) supplied at a flow rate of 10 mL min^{-1}. DSC curves were obtained using a TA Instruments Q20 calorimeter operated at a heating rate of 10 ºC min^{-1}, under an atmosphere of N_2 at a flow rate of 50 mL min^{-1}. Approximately 10 mg portions of the HDES solvents were placed in aluminum crucibles and heated from -70 to 10 ºC. The Q600 software was used to determine the onset and endset temperatures for all thermal events in the TGA and DSC curves.

2.3 Experimental design for cadmium extraction using an ultrasonic bath

The 3^3 Box-Behnken factorial experimental design consisted of three levels (-1, 0, +1), allowing the estimation of first and second order coefficients (linear and quadratic) for the fitted mathematical model, using a minimum number of experiments (15 experiments), with triplicates at the center point. Optimization of the experimental

conditions was performed by the extraction of cadmium from a sewage sludge CRM, using an ultrasonic bath. Experimental designs were implemented separately for HDES 1, which comprised acetic acid and DL-menthol, and HDES 2, which consisted of acetic acid, pyruvic acid, and DL-menthol.

The factors considered in the experimental designs were sample mass (g), volume of HDES (mL), and extraction time (min). The % recovery of cadmium was selected as the response variable, with acceptable values considered to be within the range from 80 to 110% [21]. Table 1 shows the factors, levels, and values used for the experimental design.

Table 1. Factors and values for three levels in the Box-Behnken design.

Factors	**Level -1**	**Level 0**	**Level +1**
CRM sample mass (mg)	150	225	300
Extraction time (min)	15.0	22.5	30.0
HDES volume (mL)	2.0	3.0	4.0

Further information concerning the experimental design is provided in Table S1 (Supplementary Material). Data analysis was performed using Statistica v. 12 software, adopting a confidence level of 95%.

2.4 ICP-MS analysis

Cadmium analysis were carried out employed a NexION 300X ICP-MS instrument (PerkinElmer, Shelton, USA). Conventional nebulization was used, with torch alignment and gas flow set as recommended by the equipment manufacturer. The analyses were carried out using kinetic energy discrimination (KED) mode, with testing of three different He flow rates (2.0, 2.5, and 3.0 mL min^{-1}). Calibration standards were prepared from a 1000 mg L^{-1} cadmium stock solution. For analysis of the extracted sewage, a cadmium calibration curve was constructed with working standards at concentrations in the range from 0.50 to 50 μg L^{-1}, prepared in a matrix-matched medium containing 1.0% HDES 1 (acetic acid and menthol, molar ratio 1:1).

The Box-Behnken design samples were analyzed in both standard mode and using KED, in order to establish the best methodology for the analysis. Table 2 shows all the operating conditions for the determination of cadmium in the samples by ICP-MS.

Table 2. ICP-MS operating conditions.

Parameter	**Operating condition**
Radiofrequency power (kW)	1.6
Plasma gas flow rate (L min^{-1})	18
Auxiliary gas flow rate (L min^{-1})	1.2
Nebulizer gas flow rate (L min^{-1})	1.0
Sample uptake rate (L min^{-1})	0.7
KED mode	
Gas channel	He
He flow rate (mL min^{-1})	2.0 - 3.0
Calibration range (µg L^{-1})	0.50 - 50
Nebulizer	Concentric
Spray chamber	Cyclonic
Isotope (*m/z*)	$^{114}Cd^{+}$
Number of replicates	3

2.5 Quality parameters of the methods for determination of cadmium in the samples

The linearity of the method was assessed by ANOVA (95% confidence level), using triplicate calibration curves in the concentration range 0.500-50.0 µg L^{-1}. For the limits of detection (LOD) and quantification (LOQ), the formulas 3 s/a and 10 s/a were used, where "s" is the standard deviation of ten analytical blanks and "a" is the slope of the analytical curve [21].

The accuracy and precision of the method considered the % recovery and relative standard deviation (RSD %), respectively, obtained using the sewage sludge CRM sample.

2.6 Samples

After determining the optimal extraction condition, the developed methodology was applied to the sewage sludge CRM and to the sludges from two sewage treatment stations (A and B, both in São Paulo state, Brazil), in addition to a sewage sludge from a poultry production facility (also in São Paulo state, Brazil). The sewage sludge samples were collected during the winter of 2023 (in the Southern Hemisphere), in plastic pots that had been previously decontaminated for 24 h in a 10% HNO_3 bath. The samples were dried in an oven at 60 ºC for 72 h, in accordance with EPA guidelines [22]. After drying, the samples were ground and sieved through 63 μm meshes, for subsequent use in the experiments.

3. Results and Discussion

3.1 Density and viscosity measurements

Deep eutectic solvents can be prepared by different methods, such as heating, ultrasound, microwave radiation, lyophilization, or evaporation. In this work, it was decided to use heating, since this method is simple, efficient, and allows the preparation of several DES or HDES simultaneously. Menthol was selected as one of the components, because it is nontoxic, widely available, and an excellent hydrogen bond acceptor [23].

Density and viscosity are important physicochemical properties of HDES, since they directly influence the mass transport phenomena and, consequently, the possible applications of these solvents. Solvents with low viscosity are considered ideal for use in extraction processes, as they allow greater mass transfer. When ICP-MS is used for the analysis of metals, solvents with lower viscosities favor spray formation in the nebulizer, as well as mass transport of the sample to the plasma [24].

The use of precursors rich in hydroxyl groups acts to increase the sites available for the formation of hydrogen bonds, as also observed when using organic acids, which produce solvents that are denser and more viscous. In the present case, it could be seen that the addition of pyruvic acid in the ternary mixture increased the density and viscosity of the solvent, as expected. For HDES 1, the density and viscosity values at 25 ºC were 0.930 ± 0.0051 g mL^{-1} and 5.96 ± 0.026 mPa s, respectively. For HDES 2, the values were 0.988 ± 0.0022 g mL^{-1} and 8.87 ± 0.081 mPa s, respectively.

It is known that for non-ionic HDES, as in the present case, the viscosity increases as the HBD chain increases. This was observed here, since the addition of a second HBD (pyruvic acid) in HDES 2 led to a marked increase in the viscosity of the mixture, when compared to HDES 1. A similar effect was observed in relation to density in work involving the preparation of HDES and NADES [25, 26, 27, 28].

3.2 FTIR analysis

FTIR is widely used to confirm molecular interactions, in addition to being an excellent method of characterization, enabling the confirmation of chemical functional groups and/or molecular bonds. In this way, the formation of a new HDES can be confirmed by the existence of hydrogen bonds between DL-menthol and hydrogen bond donors, which in this case were acetic acid and pyruvic acid. The structures of these hydrogen bond donors result in a characteristic band at approximately ~1700 cm^{-1}, related to the ketone or carboxylic acid groups. The hydrogen bond acceptor (DL-menthol) presents a characteristic band at approximately ~3000 cm^{-1}, related to the hydroxyl group [27]. As shown in Figure 1, the FTIR spectra can confirmed the formation of hydrogen bonds between DL-menthol and the hydrogen bond donors, with the band corresponding to the carboxylic group shifting to higher wavenumbers (~1711 cm^{-1} for the mixture of acetic acid and menthol, and ~1713 cm^{-1} for the mixture of acetic acid, pyruvic acid, and menthol). In addition, the physical state of the HDES formed (liquid and homogeneous for all the mixtures) evidenced the formation of the eutectic mixture, since the reagents were initially in solid form [27].

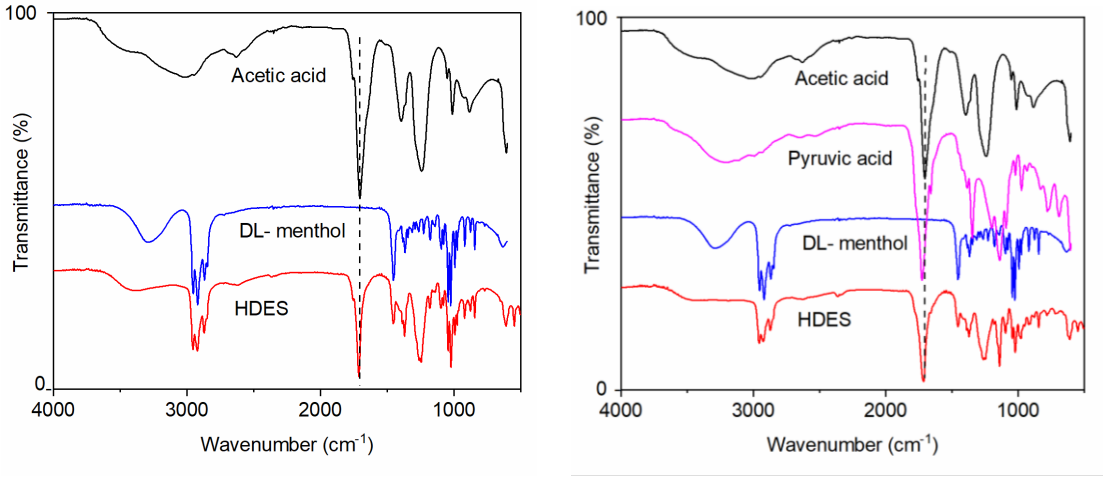

Figure 1. FTIR spectra of HDES 1 (DL-menthol and acetic acid) and HDES 2 (DL-menthol, pyruvic acid, and acetic acid).

3.3 DSC and TG/DTG measurements

The TG/DTG curves for the HDES solvents are shown in Figure 2(a-b). Pyruvic acid was added with the intention of providing greater hydrogen bond-type interactions and consequently increasing interactions between the species of interest. In this paper, for the first time the preparation of HDES from the hydrogen bond donors, acetic acid and pyruvic acid plus the acceptor DL -menthol was reported. The TG curves enabled analysis of the thermal behavior of the two synthesized solvents. It can be observed that the mass loss process for both solvents begins at low temperature values. The TG curves of HDES1 and HDES2 show a significant mass loss up to 155°C and 138°C, respectively. The DTG curves showed the presence of two slopes, with onset points at approximately 41.8 and 97.1 ºC for HDES 1, and at 41.8 and 88.3 ºC for HDES 2. The DTA curves for the solvents (Figure S1, Supplementary Material) showed the presence of two endothermic peaks, with minima at 78 and 136 ºC for HDES 1, and at 56 and 141 ºC for HDES 2. These two endothermic peaks could be attributed to the thermal decomposition of the solvents as the temperature increased.

Figure 2(c-d) displays the DSC curves obtained at low temperatures for the two synthesized eutectic solvents. These curves enabled the determination of the specific temperature at which two or more substances form a single liquid phase, leading to the formation of an ideal liquid mixture [27, 28]. This temperature is recognized as the eutectic point [27, 28]. The eutectic points for the HDES solvents were determined by

tracing two tangent straight lines on the DSC curves. The eutectic point temperatures for HDES 1 and HDES 2 were found to be -22.5 and -29.1 ºC, respectively (Figure 2c-d).

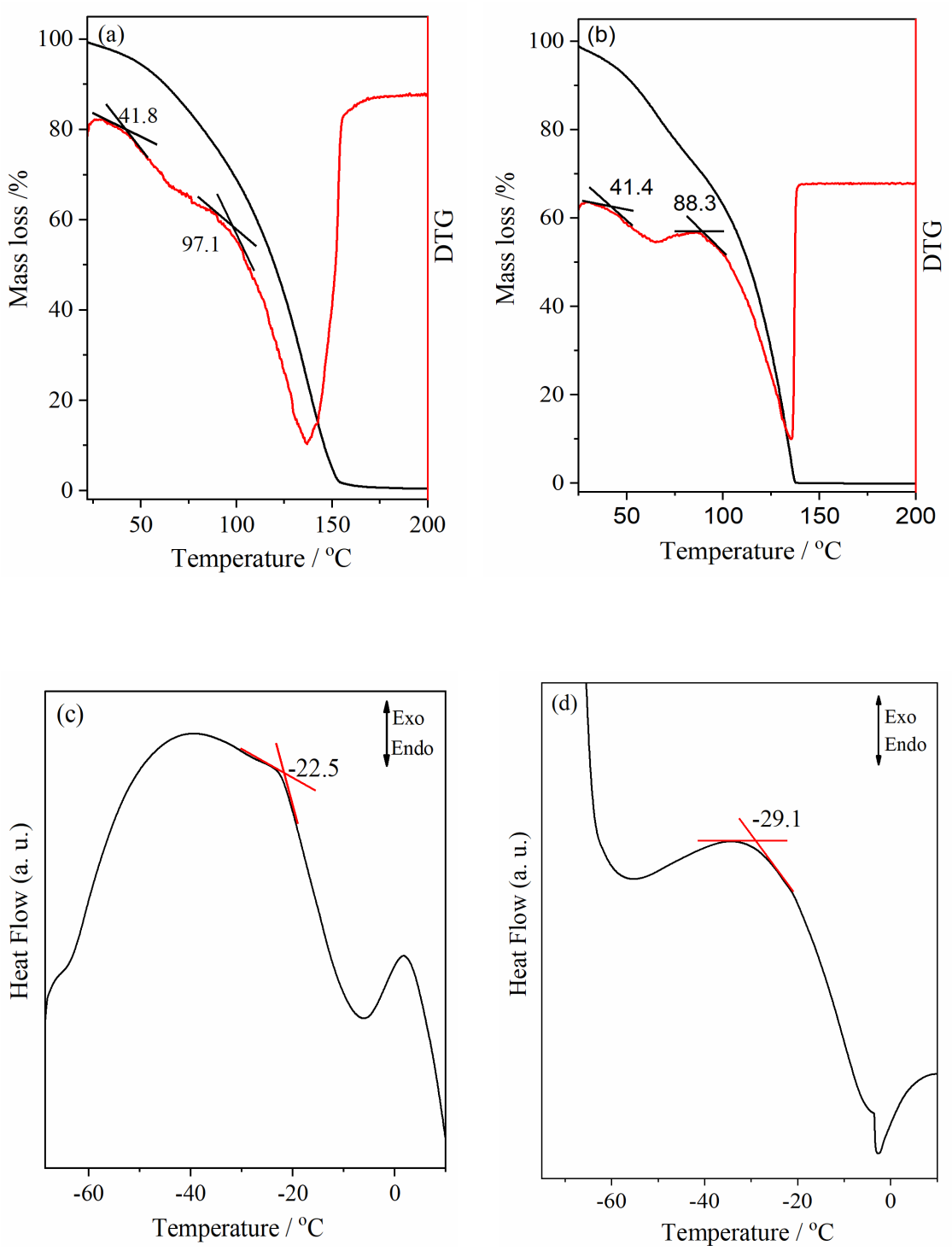

Figure 2. (a-b) TG/DTG and (c-d) DSC curves for HDES 1 and HDES 2.

3.4 Experimental design

The data obtained by ICP-MS revealed that the best analysis condition, based on the recovery results, was in KED mode, with He flow rate of 3.0 mL min^{-1}. This condition was chosen to compose the experimental design and was used at the time of sample analysis.

The results of the experimental design were analyzed separately for HDES 1 and HDES 2, with evaluation based on the recovery percentages obtained using the sewage sludge CRM. Figure 3 presents the results in the form of Pareto charts, adopting a 95% confidence level ($p > 0.05$). For HDES 1, the charts demonstrated the significance of linear (L) and quadratic (Q) variables of the mathematical model. The main effect factors included CRM mass (L), HDES volume (L), and CRM mass (Q) as independent variables, with a residual mean square (RMS) of 19.47. Comparable behavior was observed for HDES 2, where the HDES volume (L), CRM mass (L), and CRM mass (Q) were identified as main effects, with RMS of 22.18. These results indicated satisfactory model fits with first and second order coefficients.

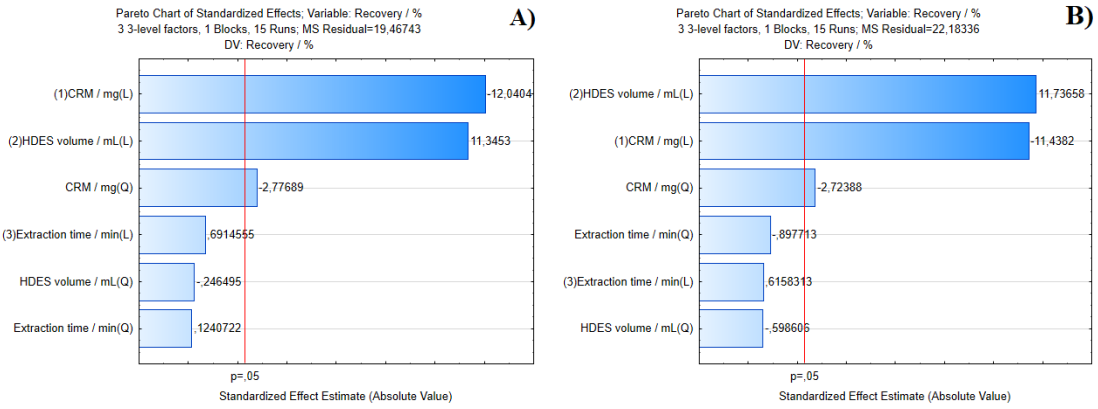

Figure 3. Pareto charts of main effects ($p > 0.05$) for the extraction and recovery of Cd in the CRM using (A) HDES 1 (acetic acid and DL-menthol) and (B) HDES 2 (acetic acid, pyruvic acid, and DL-menthol).

Application of ANOVA (Table S2, Supplementary Material) resulted in correlation coefficients (R^2) of 0.9741 and 0.9719 for HDES 1 and HDES 2, respectively, indicating excellent fits of the mathematical models. The RMS values were 19.467 and 22.183 for HDES 1 and HDES 2, respectively, demonstrating minimal residual variation in the response variable (% recovery) after applying the model. Among the selected

independent variables and their interactions, CRM mass (L) and CRM mass (Q), as well as HDES volume (Q), were identified as the most significant (p = 0.05).

Response surface graphs were plotted for HDES 1 and HDES 2, depicting the relationships between the specified two independent variables (among CRM mass, HDES volume, and extraction time) and the dependent variable (% recovery). For HDES 1, evaluation of the effects of extraction time (min) and CRM mass (mg), shown in Figure 4A, revealed that recoveries above 80% were consistently achieved for extraction times from 15 to 30 min. Notably, the optimal analysis time was 15 min, significantly reducing the ultrasonication time required to achieve satisfactory results. Considering the effect of CRM mass (mg), the best results were obtained with the lowest mass evaluated (150 mg). The surface plot for the effects of extraction time (min) and HDES volume (mL), shown in Figure 4B, revealed that the effect of time was non-significant, with recoveries above 30% obtained for times from 15 to 30 min. However, superior recoveries were observed with larger volumes of extracting solvent, particularly using HDES 1, with volumes exceeding 3.0 mL. For the relationship between CRM mass and HDES volume (Figure 4C), it was observed that lower CRM mass values (ranging from 150 to 200 mg) enabled recoveries above 80%, with HDES volumes exceeding 2.5 mL. These results were associated with satisfactory curvatures of the response surfaces obtained for the use of HDES 1 composed of acetic acid and DL-menthol.

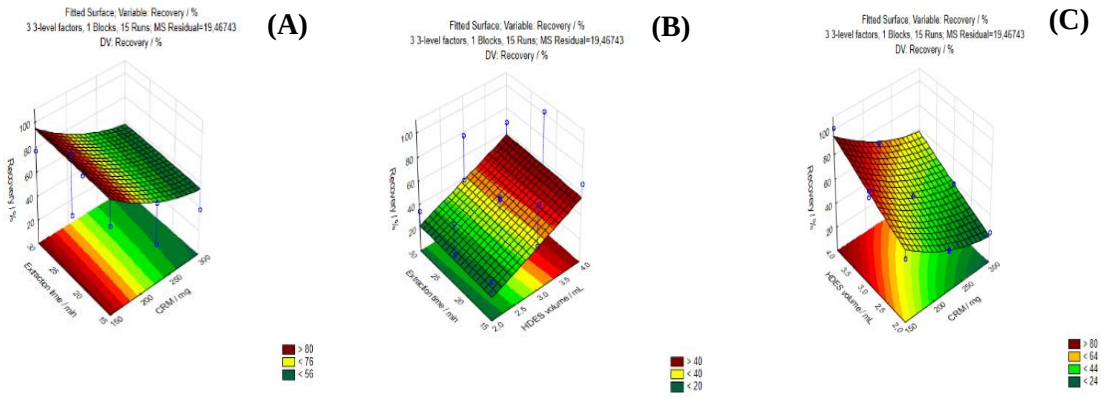

Figure 4. Response surface plots for the effects of the independent variables on % recovery of Cd from the CRM using HDES 1 (acetic acid and DL-menthol): (A) CRM mass (mg) and extraction time (min); (B) extraction time (min) and HDES volume (mL); (C) HDES volume (mL) and CRM mass (mg).

The response surface plots for HDES 2 exhibited behaviors analogous to those observed for HDES 1. The plot obtained with the variables extraction time and CRM mass (Figure 5A) showed that recoveries of 100% were achieved with a smaller CRM mass, ranging between 150 and 180 mg, with time being insignificant. An extraction time of only 15 min was sufficient to achieve satisfactory results. Considering the HDES volume and extraction time (Figure 5B), recoveries exceeding 60% were achieved with larger HDES volumes in the range from 3.0 to 4.0 mL, regardless of the analysis time. Finally, Figure 5C shows that recoveries above 100% could be achieved with CRM mass between 150 and 200 mg and HDES 2 volume between 3.0 and 4.0 mL. Excellent recovery results were observed for HDES 2, with HDES volume between 3.0 and 4.0 mL, regardless of the extraction time, allowing selection of an extraction time of only 15 min in this ultrasound- assisted method. All the response surface graphs presented favorable curvature.

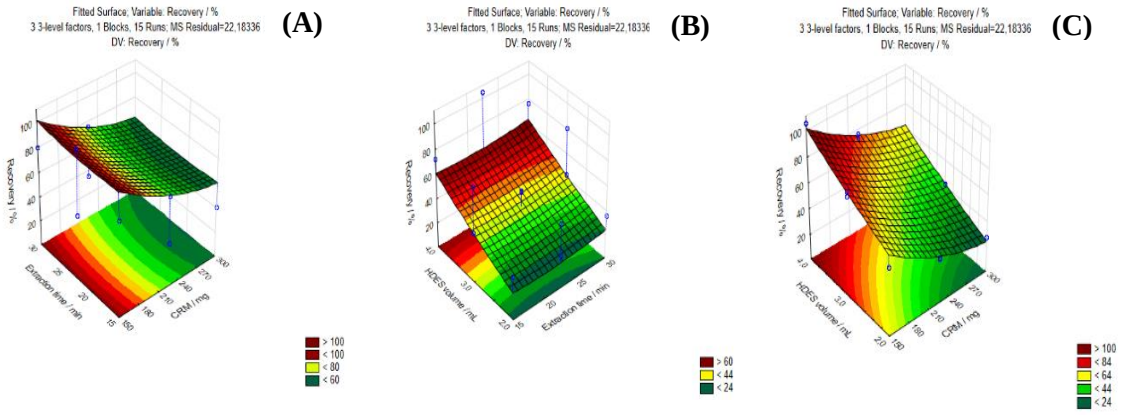

Figure 5. Response surface plots for the effects of the independent variables on % recovery of Cd from the CRM using HDES 2 (acetic acid, pyruvic acid, and DL-menthol): (A) extraction time (min) and CRM mass (mg); (B) HDES volume (mL) and extraction time (min); (C) HDES volume (mL) and CRM mass (mg).

The contour plot data supported the results obtained previously, enabling definition of the optimal conditions for application of the developed methodology. Higher accuracies (101% for HDES 1 and 104% for HDES 2) were obtained using higher HDES volume (4.0 mL) and lower sample mass (150 mg). Therefore, the condition considered optimal was HDES volume of 4.0 mL, sample mass of 150 mg, and extraction time of 15.0 min.

3.5 Quality parameters of the proposed methods

The linearity of the method was evaluated by constructing a calibration curve in the concentration range 0.500-50.0 μg L^{-1}, which could be described by the following equation: y = 1216[Cd] – 939.7; R^2 = 0.999. Application of ANOVA showed no lack of fit for the method (F_{cal} (1.91 x 10^{-12}) < $F_{tab\ 95\%}$ (10.9 x 10^3)). The LOD and LOQ values were 0.271 and 0.905 μg L^{-1} (HDES 1), and 0.280 and 0.933 μg L^{-1} (HDES 2), respectively (Table 3). The precision (RSD %) of the method was determined as the repeatability of the measurements (n = 3), obtaining values of 2.65% (HDES 1) and 2.34% (HDES 2) (Table 3).

Table 3. Figures of merit for analysis of Cd by UAE/ICP-MS (n = 3) methodology with extraction using HDES 1 and HDES 2.

Analyte	HDES 1			HDES 2		
	LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)	LOD (μg L^{-1})	LOQ (μg L^{-1})	Recovery (%)
^{114}Cd	0.271	0.905	101	0.280	0.933	104

Cadmium was determined in the sewage sludge and CRM samples using external calibration. The concentrations obtained for sewage sludges A and B and the poultry sewage are summarized in Table 4.

Table 4. Concentrations of Cd in the samples analyzed by the UA/ICP-MS methodology with extraction using HDES 1 and HDES 2.

Analyte	HDES 1				HDES 2			
	Sewage sludge A	Sewage sludge B	Poultry sewage sludge	CRM	Sewage sludge A	Sewage sludge B	Poultry sewage sludge	CRM
^{114}Cd	2.073 ± 0.10[a]	2.707 ± 0.20[a]	6.023 ± 0.84[a]	1.920 ± 0.21[a]	2.103 ± 0.11[a]	2.067 ± 0.23[a]	5.319 ± 0.14[a]	1.981 ± 0.16[a]

[a]Average (μg g^{-1}) ± standard deviation (n = 3).

In the case of these types of samples, it should be noted that environmental variations can cause compositional differences, influenced by factors including climate, season, and anthropic activity. The analytical Eco-Scale method was used to evaluate the HDES preparation procedure. In this technique, a value of 100 is the ideal score, with penalty points being deducted for each parameter that differs from the ideal value (in other words, penalty points are assigned for aspects of methods that do not comply with the principles of green chemistry). The energy consumption for the HDES preparation was calculated, resulting in a value of 0.0392 kWh mL^{-1} for each mixture. The only penalty occurred due to the use of agitation and heating, for which 2 points were deducted. According to Van Aken et al. [29], an Eco-Scale score above 75 represents a green method. Here the HDES preparation method received a score of 98, out of a total of 100 possible points, representing excellent green characteristics [30].

The greenness of the cadmium extraction and determination was evaluated using the AGREE open-source software. This generates a graph with scores that can vary from 0 (lowest greenness) to 1 (highest greenness), based on the 12 principles of green analytical chemistry (GAC) [29]. The graph generated by the software shows a color scale ranging from red to yellow to green, indicating weak aspects of the method (red color) and its strong points (green color). The items described in Figure 6 were evaluated using AGREE, obtaining a final score of 0.7 (without considering the energy consumption of analyzes by ICP-MS), indicating that the developed method could be considered green.

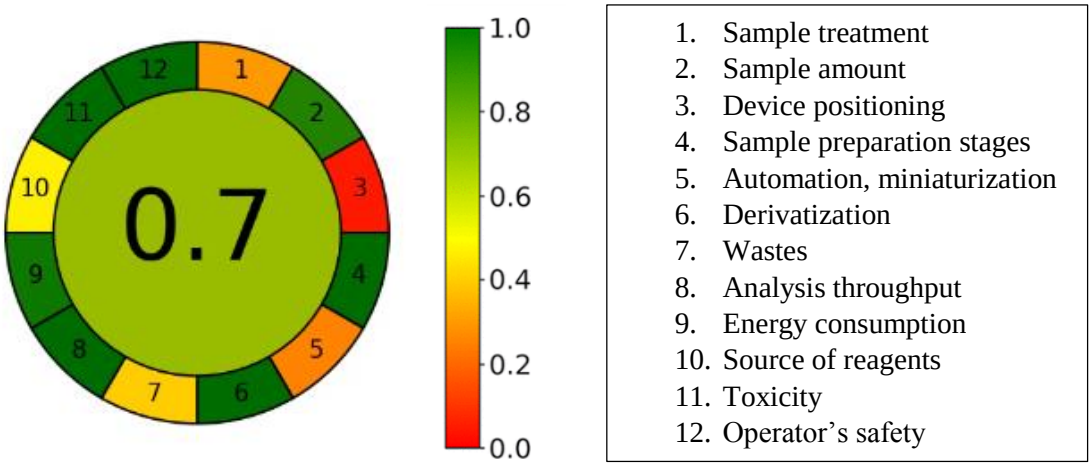

Figure 6. Use of AGREE to evaluate the greenness of the ultrasound-assisted extraction method.

Previous studies have reported the use of HDES prepared from DL-menthol and acetic acid for the extraction of pesticides from aqueous environments [11], the extraction of bisphenol A from aqueous medium [32], and the removal of diclofenac [33], but to date no reports were found concerning the use of this HDES for the removal of cadmium from sewage sludge. The use of HDES prepared using acetic acid, pyruvic acid, and DL-menthol has not previously been reported for any application (including the extraction of cadmium in sewage sludge).

The extraction of cadmium present in sewage sludge is generally performed using acidic solutions (HNO_3), followed by ICP-MS or ICP OES analysis [34,35]. The Table 5 compares the recoveries of cadmium extractions in sewage sludge samples using acidic solutions and the method developed in this paper. The high viscosity of HDES did not cause any impact on the analyses, as it was not used purely at the time of the analyses, it was diluted for use in the ICP (100x dilution) so as not to cause instrumental problems. It should be noted that the use of acidic solutions and microwaves are official methods for preparing samples, although a disadvantage is that they produce toxic residues, in addition to requiring long extraction times that compromise the analytical frequency.

Table 5. Comparison of cadmium extraction efficiency in sewage sludge samples using different solutions

LOD (µg L^{-1})	LOQ (µg L^{-1})	Amount of sample and reagents	Extracting solution viscosity (mPa s)	Recovery (%)	Instrumental apparatus	Refer.
4.60	15.4	0.5 g/ 10 mL HNO_3	0.76	>95	ICP- OES	35
*Nd	*Nd	0.5 g/ 25 mL polyepoxysuccinic acid	*Nd	78	ICP- OES	36
*Nd	*Nd	1g/ 5.0 mL HNO_3 /2.0 mL HCl/ 3.0 mL HF	*Nd	101	ICP-MS	37
0.271	0.905	150 mg/ 4.0 mL HDES (DL-menthol and acetic acid)	5.96	101	ICP-MS	This paper
0.280	0.933	150 mg/ 4.0 mL HDES (DL-menthol, acetic acid, and pyruvic acid)	8.87	104		

* Nd= not described

Conclusions

Two different mixtures containing DL-menthol were prepared by stirring and heating, for subsequent use in the extraction of cadmium from different sewage sludge samples. The mixtures were evaluated using FTIR, DSC, viscosity, and density analyses, and proved to be equally efficient in removing the selected analyte. Box-Behnken experimental design was used for elucidation of the best HDES volume, sample mass, and extraction time to be used in the analysis. The analytical Eco-Scale method was used to evaluate the HDES preparation method, showing that the method could be considered a green technique that complied with the principles of green chemistry. The cadmium values obtained using HDES 1 and HDES 2 with sewage sludge A were 2.073 ± 0.10 µg g^{-1} (HDES 1) and 2.103 ± 0.11 µg g^{-1} (HDES 2). For sewage sludge B, the values obtained were 2.707 ± 0.20 µg g^{-1} (HDES 1) and 2.067 ± 0.23 µg g^{-1} (HDES 2). Finally, the values obtained with the poultry sewage sludge sample were 6.023 ± 0.84 µg g^{-1} (HDES 1) and

5.319 ± 0.14 μg g^{-1} (HDES 2). The sample preparation time was short (15 min), which provided high analytical frequency. Use of the prepared HDES and the ultrasound-assisted extraction method proved to be an excellent option for the extraction of cadmium from different sewage sludges, as a substitute for the nitric acid solution and microwave treatment conventionally employed in the sample preparation step.

Acknowledgments

The authors are grateful for the financial support provided by the São Paulo State Research Foundation (FAPESP, grants #2021/14581-1, #2021/14759-5, #2023/04832-2, #2019/22113-8, #2017/18531-3, and #2014/50945-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant #465571/2014-0 and grant #403839/2023-8), and Instituto Nacional de Tecnologias Alternativas para Detecção, Avaliação Toxicológica e Remoção de Micropoluentes e Radioativos (INCT-DATREM). Sara C. Cunha acknowledges FCT for the 2022.07841.CEECIND/CP1724/CT0014 contract. The LAQV-REQUIMTE authors thanks FCT Fundação para a Ciência e a Tecnologia through national funds to the project CleanH20 2022.03009.PTDC

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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.

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Declaration of interests

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