



EXPERIMENTAL AND THEORETICAL OPTIMIZATION OF EMULSIFICATION LIQUID-LIQUID MICROEXTRACTION FOR DETERMINATION OF HERBICIDES USING FATTY ACID DEEP EUTECTIC SOLVENTS

(Pengoptimuman Secara Eksperimen dan Teori bagi Pengemulsi Mikroekstraksi Cecair-Cecair untuk Penentuan Racun Perosak Menggunakan Pelarut Eutektik Terdalam Asid Lemak)

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Abstract

Fatty acid deep eutectic solvents (DES) became known as a promising alternative to traditional organic solvents for various extraction processes. In this study, a hydrophobic DES comprising a mixture of lauric acid (C_{12}) and pelargonic acid (C_9) at a molar ratio of 1:3, was utilized to extract 2,4-D and MCPA herbicides from aqueous solutions. FTIR and TGA were utilized in order to characterize the synthesized DES. Emulsification liquid-liquid microextraction (ELLME) technique is employed to enhance the efficiency of extraction. Furthermore, response surface methodology (RSM) was applied to optimize the extraction process of these herbicides while considering parameters such as volume of DES and emulsifying agent and pH. RSM method has various advantages, including its simplicity, fast, low cost, and great extraction efficiency. It also helps in selecting the optimum condition method to analyze the relationship between the variable, where pH 2, 265 μL volume of DES and 200 μL volume of tetrahydrofuran (THF) were the optimal conditions obtained in this study. Under these conditions, the coefficients of determination (R^2) were obtained between 65% and 79%. In addition, the results obtained from the experiment are comparable to or closely aligned with RSM, hence there was a good fit between the two methods. These results revealed that the fatty acid-based DES demonstrated remarkable efficiency in extracting 2,4-D and MCPA herbicides as well as contributing to the development of environmentally friendly extraction techniques.

Keywords: response surface methodology, 2,4-Dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, hydrophobic DES, emulsification liquid-liquid microextraction, pesticides

Abstrak

Pelarut eutektik terdalam asid lemak (DES) dikenali sebagai alternatif kepada pelarut organik tradisional untuk digunakan dalam pelbagai proses pengekstrakan. Dalam kajian ini, DES hidrofobik yang terdiri daripada campuran asid laurik (C_{12}) dan asid pelargonik (C_9) pada nisbah molar 1:3, telah digunakan untuk mengekstrak racun herba 2,4-D dan MCPA daripada larutan akueus. FTIR dan TGA telah digunakan untuk mengenalpasti DES yang disintesis. Teknik pengekstrakan mikro cecair-cecair pengemulsi

(ELLME) digunakan untuk meningkatkan kecekapan pengekstrakan. Tambahan pula, metodologi permukaan tindak balas (RSM) digunakan bertujuan untuk mengoptimumkan proses pengekstrakan racun herba ini dan mempertimbangkan parameter seperti isipadu DES dan agen pengemulsi dan pH. Kaedah RSM ini mempunyai pelbagai kelebihan, termasuk kesederhanaan, pantas, kos rendah, dan kecekapan pengekstrakan yang hebat. RSM juga membantu dalam memilih keadaan kaedah yang optimum untuk menganalisis hubungan antara pembolehubah, di mana pH 2, 265 μL isipadu DES dan 200 μL isipadu tetrahydrofuran (THF) adalah keadaan optimum yang diperolehi dalam kajian ini. Dengan keadaan ini, pekali penentuan (R^2) diperolehi antara 65% dan 79%. Di samping itu, keputusan yang diperolehi daripada data eksperimen adalah setanding atau sejajar rapat dengan data RSM, oleh itu terdapat kesesuaian yang baik antara kedua-dua kaedah. Keputusan ini mendedahkan bahawa DES berasaskan asid lemak menunjukkan kecekapan yang luar biasa dalam mengekstrak racun herba 2,4-D dan MCPA serta menyumbang kepada pembangunan teknik pengekstrakan mesra alam.

Kata kunci: metodologi permukaan tindak balas, 2,4-Dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, DES hidrofofik, pengekstrakan mikro cecair-cecair pengemulsi, racun herba

Introduction

Providing a safe and healthy environment for everyone while concurrently reducing the hazardous effects of current pollutants is one of the major challenges encountered by modern industry. Pesticides account for 9 of the top 12 most harmful and persistent pollutants identified by the Stockholm Convention on Persistent Organic Pollutants [1]. Herbicides, insecticides, fungicides, and other pesticides are used extensively in agriculture, and as a result, these compounds are dispersed in the environment in large quantities [2]. Therefore, the presence of pesticide residues in water, soil, and agricultural products has the potential to surpass the permitted thresholds, resulting in significant environmental contamination [2] and even human health problems [3]. Phenoxy acid herbicides are extensively employed in agricultural practices for the purpose of managing the proliferation of undesirable weeds [4]. These compounds' cost-effectiveness and high efficacy at low concentrations are widely acknowledged, albeit accompanied by potential hazards to human health [5]. Following application, these herbicides have the potential to readily infiltrate water systems as a result of their high-water solubility. They are known to have mutagenic, teratogenic, and carcinogenic properties that can lead to cytotoxicity and DNA damage after extended exposure. Detecting these substances in food and environmental samples raises significant concerns regarding their potential impact on human and animal consumption [6]. Hence, it is imperative to closely monitor the presence of these herbicides in both food and environmental samples to ensure adherence to established environmental quality standards.

Concerned with the safety of the environment and people's health, experts have been working hard to find sensitive and careful ways to test for pesticides. In order to determine these pesticides, a sample pre-treatment plays an important role during the analytical procedure. The techniques used for sample pre-treatment revolve mainly around extracting analytes from the sample matrix into a suitable solvent for the sample to be pre-concentrated and cleaned. Conventional sample pre-treatments such as liquid-liquid and solid phase extraction are commonly employed for pre-concentration of phenoxy acid herbicides [4, 6]. The use of these procedures is commonly hindered by labour and cost intensiveness, as well as the substantial consumption of toxic solvents, hence rendering these techniques incongruent with sustainable development objectives.

Microextraction techniques have emerged as a recent approach for the analysis of pesticides in environmental and food samples. These techniques have been chosen for their miniaturization, simple operation, low cost, and minimal sample and solvent consumption [3]. Solid phase micro-extraction (SPME), magnetic solid phase extraction (MSPE), emulsification liquid-liquid microextraction (ELLME), and dispersive solid phase extraction (DSPE) are the most frequently used pre-treatment methods for analysing pesticides in food samples and environmental water [7]. Out of these techniques, ELLME stands out as a widely utilized pre-treatment method for pesticide residues [7] due to its simplicity, ease of operation, rapidity, and high extraction efficiency [8]. In ELLME, sample solutions are treated with extraction solvents of high solubility

and emulsifier solvents to extract the target analytes. Among the plethora of microextraction techniques, ELLME offers several advantages, such as small amount of organic solvent consumption, time savings, and greater extraction efficiency [9].

For the development of ELLME conditions, organic solvents must be used in the determination of various types of pesticides. However, a massive number of organic solvents contribute to negative impacts on human health and the environment. The advent of green chemistry has led to the proposal of deep eutectic solvents (DES), a novel group of environmentally friendly solvents, as a potential substitute for traditional solvents, due to their green features [10]. Usually, DES is made from economical materials using simple preparation steps. It is ideal for microextraction methods due to its great thermal stability and biodegradability features. In its typical form, DES is produced by combining two substances, a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), which are then hydrogen-bonded to one another to produce a molecule with a low melting point [11].

Recently, researchers have proposed the utilization of hydrophobic deep eutectic solvents (DES) to extract analytes from water samples. Hydrophobic DES are formed by combining one or two hydrophobic components, resulting in DES with hydrophobic nature. Consequently, researchers have directed their attention towards the potential of hydrophobic deep eutectic solvents (DES) derived from fatty acids. Previous studies have demonstrated the efficacy of hydrophobic deep eutectic solvents (DES) as extraction media for a diverse range of analytes. Florida et al. initially reported the utilization of hydrophobic DES synthesis by combining two fatty acids, HBA and HBD, to remove bisphenol A [12]. Subsequently, DES derived from fatty acids was used for harmful industrial dye removal [13] in addition to the recognition of benzophenone and salicylate UV filters [14].

Additionally, it is crucial to optimize the extraction process in analytical methods to minimize the utilization of materials, decrease process costs, and streamline time-consuming steps. In this context, optimization

studies involving chemometric modelling hold significance. Response surface methodology (RSM) is a computational approach to optimizing intricate processes [15]. The statistical technique employed in this study aims to optimize the values of a dependent variable by considering multiple independent factors. Its purpose is to facilitate a comparison between theoretical predictions and empirical observations [16]. The RSM method enables the efficient testing of all variables associated with consumer evaluation within a condensed timeframe during the optimization process. This enhances the efficiency of the laboratory testing procedure. Consequently, this leads to a decrease in expenses, utilization of resources, and the requisite number of experimental trials [11], [16]. In addition to these, using quantitative data in the context of Response Surface Methodology (RSM) enables the evaluation of relationships and interactions among multiple parameters. This facilitates the identification of the most favorable conditions in an experiment. Additionally, mitigating matrix impact and enhancing analyte extraction efficiency are supplementary benefits [11].

In light of the explanations mentioned above, this study aimed to synthesize a deep eutectic solvent composed of fatty acids, specifically lauric acid and pelargonic acid. Additionally, the fatty acids DES was analysed characterized by using thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR). Moreover, two types of herbicides, 2,4-D and MCPA were extracted by using this DES. To achieve the best extraction effect, the application of response surface methodology (RSM) was employed to determine the optimum conditions by considering a few important variables (volume of DES, volume of emulsifier solvent, and pH).

Materials and Methods

Materials

All the pesticides used namely MCPA and 2,4-D (purity, $\geq 98.0\%$) were purchased from Sigma-Aldrich (St Louis, MO, USA). Lauric acid (purity $\geq 98.0\%$) and pelargonic acid (purity, $\geq 97.0\%$) were also supplied by Sigma-Aldrich (St Louis, MO, USA). Hydrochloric acid (37%) and acetonitrile were obtained from QRec (Auckland, New Zealand). Tetrahydrofuran (analytical

grade) was purchased from Merck (Darmstadt, Germany). The herbicide stock solutions were prepared in acetonitrile at a concentration of $1000 \mu\text{g mL}^{-1}$. The stock solutions of each herbicide were serially diluted in acetonitrile to create working solution combinations of $10 \mu\text{g mL}^{-1}$.

Instrumentations

The FTIR spectra were recorded using Perkin Elmer 2000 FTIR instrument (Waltham, MA, USA) to verify functional groups present in the synthesized DES. The spectra were obtained during 16 scans within the range of $400\text{--}4000 \text{ cm}^{-1}$. Thermogravimetric analysis (TGA) was performed using Perkin Elmer STA 6000 (Waltham, MA, USA) under 5 mL min^{-1} nitrogen flow, at temperature ranging from 30 to 600°C and heating rate of $10^\circ\text{C min}^{-1}$. UV-2600 Spectrophotometer (Shimadzu, Japan) was used to determine concentration of herbicides in sample by using standard quartz cells with a path length of 1 cm. The pH value was measured with a HANNA HI 5221-02 Laboratory Research Grade Benchtop pH/mV Meter with 0.001 pH resolution.

Preparation of DES

The DES was synthesized in the same way as in the previous study by Florindo et al. [12] by combining two distinct fatty acids in a sealed glass vial and it was heated in a water bath at 40°C for 30 minutes. After synthesis, a homogeneous and clear solution was observed. The DES used in this work was prepared with the following ratio: Lauric acid: Pelargonic acid (1:3).

Emulsification liquid-liquid microextraction

In this work, 10 mL of aqueous solution containing targeted analytes ($1.0 \mu\text{g mL}^{-1}$) adjusted to pH 4 to 6 using 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solutions was placed in 15 mL centrifuge tube. To extract the targeted analytes, DES and tetrahydrofuran were added to the aqueous solution with a micropipette. A cloudy solution was observed after the addition of emulsifier solvent (THF) into the extraction mixture which initiates the process of the aggregation and emulsification of DES. Subsequently, the extraction process was conducted using ultrasound in an ultrasonic bath for a duration of 15 minutes. The targeted analytes were removed to the DES phase as the aggregated DES

dispersed into small droplets. After 10 minutes of centrifugation at 4000 rpm, two-phase separation was observed. The upper aqueous phase was extracted using a syringe, while the bottom layer was collected. The measurements for MCPA and 2,4-D were made at 290nm and 230nm, respectively using UV-Vis Spectrophotometer.

Response surface methodology

Response surface methodology (RSM) is a method employed to optimize chemical reactions to achieve the desired outcome. The experimental design was a Box-Behnken design (BBD) for three independent factors. The volume of DES, volume of THF, and pH of solution are three independent factors influenced the microextraction. Initially, physical experiments were conducted by systematically manipulating the experimental parameters to obtain the corresponding response values. Subsequently, the relationship between the input parameters and responses was established using a second-order polynomial or exponential function. Lastly, through the examination of surface graphs and the utilization of ANOVA, the optimal points were identified. Minitab Statistical Software, version 21 (Minitab Inc., United States) was employed to perform the BBD design and statistical analysis.

Results and Discussion

Characterization of DES: FTIR analysis

The synthesis of hydrophobic DES involved the combination of two fatty acids, namely lauric acid and pelargonic acid, in a molecular ratio of 1:3. As shown in Figure 1, The confirmation of the existence of functional groups in the synthesized DES was achieved by the utilization of FTIR analysis. The spectral data for each sample was obtained via 16 scans at a resolution of 4 cm^{-1} . Data was collected at wavenumbers from 500 to 4000 cm^{-1} .

The peaks were found in the range of 2850 to 2855 cm^{-1} and 2911 to 2925 cm^{-1} which attributed to the CH_2 and CH_3 groups, respectively, represented the fatty acids. Other significant peaks were observed at 1688 and 1705 cm^{-1} , which correspond to the stretching mode of the $\text{C}=\text{O}$ group. Then, a broad absorption band was identified as the hydroxyl group (O-H) of the fatty acids, as

detected within the range of 3000 cm^{-1} and 3500 cm^{-1} . This phenomenon occurs when the O-H stretching absorption of these dimers exhibits significant intensity and broadness, primarily due to the influence of hydrogen bonding. The O-H bond demonstrates a propensity to oscillate at different frequencies and positions, resulting in enhanced band elongation [17]. The presence of O-H stretch was also detected between

1410 cm^{-1} and 1426 cm^{-1} and 926 cm^{-1} and 940 cm^{-1} . The possible DES structure from the combination of lauric acid and pelargonic acid with molar ratio of 1:3 can be predicted as in Figure 2. In this DES structure, pelargonic acid acts hydrogen bonding donor, while lauric acid acts as hydrogen bonding acceptor. Therefore, the presence of these notable peaks proves that DES was successfully synthesized.

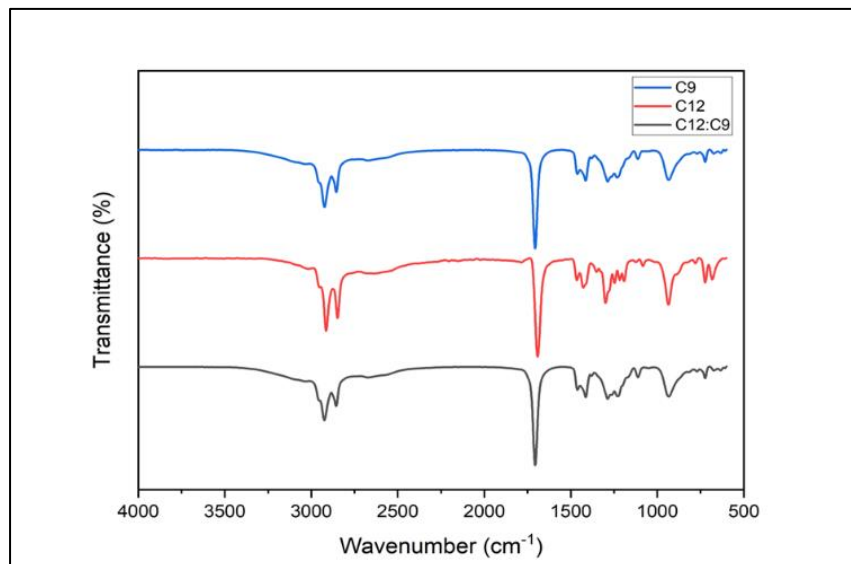


Figure 1. FT-IR spectra of the DES ($C_{12}:C_9$) and its individual components: C_{12} (lauric acid) and C_9 (pelargonic acid).

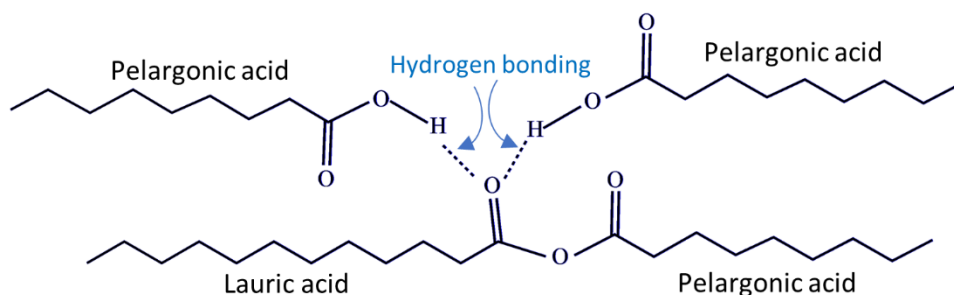


Figure 2. The possible structure of synthesized DES (combination of lauric acid and pelargonic acid with molar ratio (1:3))

Thermogravimetric analysis

As shown in Figure 3, thermogravimetric analysis (TGA) was utilized to examine the thermal properties of the synthesized DES. The TGA curve illustrates a single thermal decomposition (Td) temperature for each sample. Lauric acid, C_{12} exhibited a weight loss at

$190.03\text{ }^{\circ}\text{C}$ while pelargonic acid, C_9 showed at $166.58\text{ }^{\circ}\text{C}$. The weight loss for DES began at $189.97\text{ }^{\circ}\text{C}$. The thermal decomposition (Td) temperature determines the maximum temperature at which DES can remain liquid without decomposing. The observation of a high Td temperature indicates that a substantial amount of

energy is required to disrupts the intermolecular hydrogen bonding among the fatty acids present within the DES. Therefore, this temperature range significantly influences the applicability of DES as a solvent [18, 19]. After 200°C, the DES, and its starting materials,

decomposed rapidly. There was no further degradation detected at temperatures above 200°C. This study demonstrates that DES containing lauric acid and pelargonic acid is considered thermally stable by having a high T_d temperature.

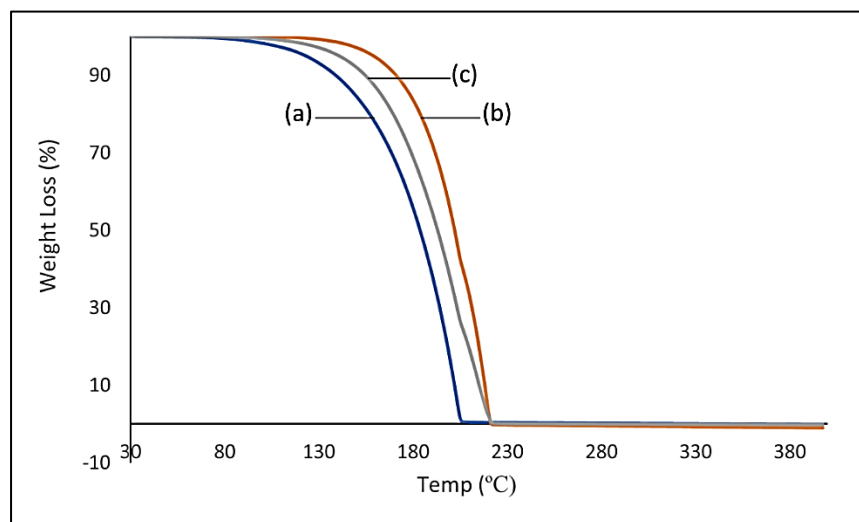


Figure 3. Thermal decomposition of the synthesized DES (C₁₂:C₉) and its raw material. (a) pelargonic acid, (b) lauric acid, (c) DES (C₁₂:C₉)

Experimental optimisation of ELLME: Effect of volume of DES

The first consideration in determining the amount of the extraction solvent should be to achieve a high level of selectivity to facilitate the effective extraction of the desired analytes. Additionally, it is crucial to ensure that the solvent has minimum solubility in water [20, 21]. The DES in this work utilized a combination of lauric acid and pelargonic acid with ratio of 1:3 as described by Sazali et al. [22]. From the previous study, DES (C₁₂:C₉) exhibited highest extraction for MCPA and 2,4-D compared to other DES. The extraction capacity of this DES is influenced by the ability of fatty acids in DES to form hydrogen bonding interaction with the analytes. This phenomenon is attributed to the DES's low viscosity and high diffusivity properties. The main interaction occurred between the analytes and DES are primarily governed by intermolecular hydrogen bonding and hydrophobic interaction, which can be attributed to the hydrophobic nature of DES [12]. For optimal results in emulsification microextraction, an appropriate

volume of extraction solvent must be used during the process [22]. Figure 4 showed the optimal volume of DES for MCPA was observed to be 500 µL. In contrast, for 2,4-D the optimal extraction volume is determined to be 30 µL of DES. However, it was observed that an increase in volume resulted in a decrease in the extraction efficiency of 2,4-D. The decrease in solubility of analytes within the DES phase can be ascribed to the dilution effect, which occurs when the volume of DES is increased. As a result, the loss in solubility ultimately results in a corresponding decrease in extraction efficiency [22, 24]. Contrarily, the situation differs for MCPA, as it requires a higher volume of DES. This is because the fatty acid DES used may have a high affinity for these analytes, enabling their efficient extraction [24]. Therefore, a higher volume of DES, up to a maximum of 500 µL, may be necessary to accommodate the MCPA herbicides effectively. Therefore, 500 µL and 30 µL volume of DES were chosen for MCPA and 2,4-D, respectively, for further experiments.

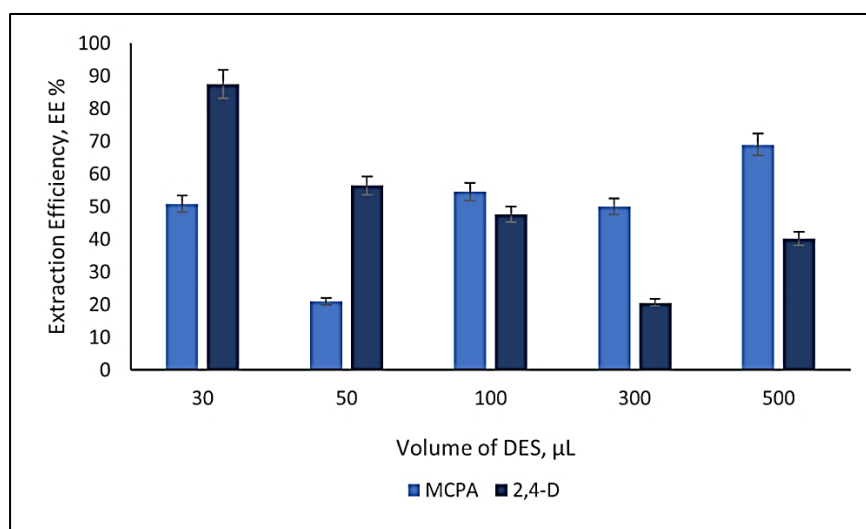


Figure 4. Effect of volume of DES. Condition: volume of THF: 500 μL ; pH 4

Effect of volume of THF

The ELLME-DES approach involves the addition of an aprotic solvent as an emulsifier, resulting in the self-aggregation and subsequent separation of the DES molecules in the aqueous phase. Indeed, the emulsifier solvent tends to exhibit favourable interactions with water molecules than the DES. As a result, the DES exhibits self-aggregation, forming minute droplets that significantly enhance the contact surface area. This process facilitates the efficient mass transfer of the analytes from the aqueous phase to the DES phase [26, 27].

In this part, the impact of emulsifier solvent volume was studied by adjusting THF volume from 200 to 900 μL . Figure 5 shows the optimum value of THF was determined to be 500 μL and 200 μL for MCPA and 2,4-D, respectively. A potential reason for the observed low THF volume was achieved for 2,4-D could attributed to the high solubility of 2,4-D in aqueous phase. However, increasing the volume of THF leads to a decrease in extraction efficiency. The occurrence can be attributed to the high concentration of emulsifier, which significantly increases the solubility of both the extractant and analytes in the aqueous phase. Consequently, their distribution into the organic phase diminishes during extraction [9]. Furthermore, phenoxy acid herbicides, such as 2,4-D and MCPA, possess notable polarity and exhibit good solubility in water,

thereby occasionally posing challenges in their precise extraction [28, 29]. To optimize the extraction process, it is important to control the volume of THF to maintain a sufficient concentration of the target analytes in the organic phase while minimizing their solubility in the aqueous phase. Thus, the optimal volume of emulsifier solvent was found to be 500 μL for MCPA and 200 μL for 2,4-D for further analysis.

Effect of pH solution

The pH of the sample solution is a crucial parameter as it influences the degree of ionization as well as the species of the analytes. Ionized forms of the target analytes may significantly affect the extraction efficiency [29]. In this study, the extractions were conducted at varying pH levels, from 2 to 10 adjusting using hydrochloric acid or sodium hydroxide solutions at 0.1 M. Herbicide extraction efficiency appears promising in an acidic environment (pH 2–6), as seen in Figure 6. The presence of herbicides such as MCPA and 2,4-D in their neutral form is primarily attributed to their inherent characteristics observed under acidic conditions. The effectiveness of partitioning into the hydrophobic DES is facilitated by their acidic nature [30]. The electronegative atoms of 2,4-D and MCPA form hydrogen bonds with the hydrogen bond donors (HBD) in their neutral state, thereby enhancing the solubility of the analytes in the deep eutectic solvent (DES) phase. The pH of the sample solutions was

adjusted to an acidic level for the subsequent investigations, as previous research has indicated that

acidic conditions are optimal for the efficient extraction of most herbicides.

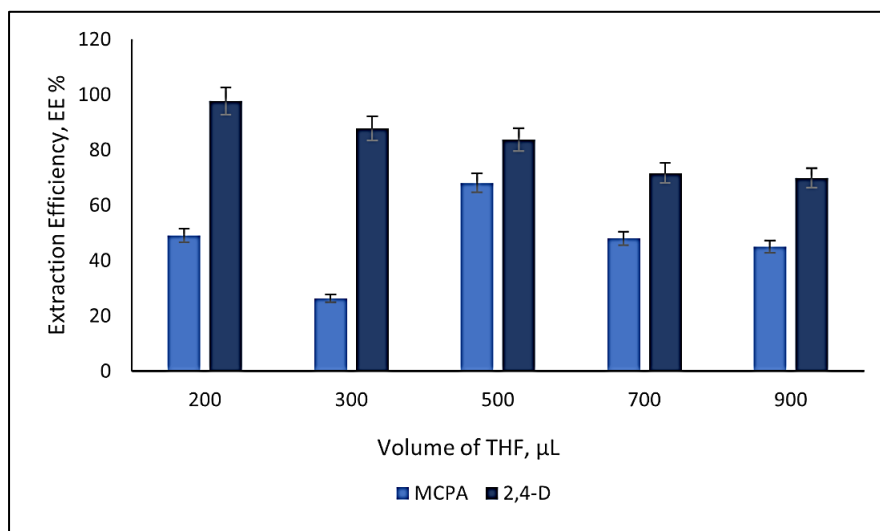


Figure 5. Effect of Volume of THF. Condition: volume of DES: 30 μL (2,4-D), 500 μL (MCPA); pH 4

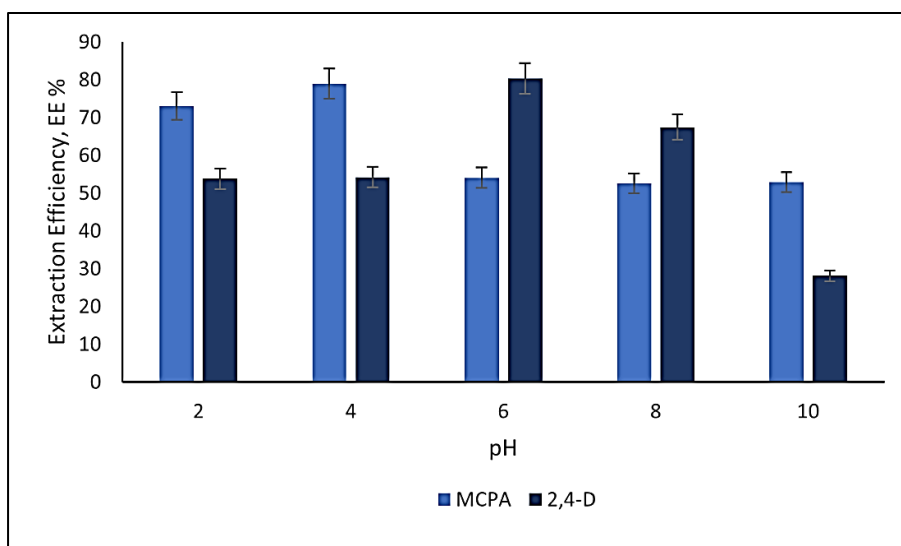


Figure 6. Effect of pH. Condition: volume of DES: 30 μL (2,4-D), 500 μL (MCPA); volume of THF: 200 μL (2,4-D), 500 μL (MCPA)

Theoretical optimization of ELLME using response surface methodology (RSM)

Response surface methodology (RSM) is a prominent statistical and mathematical technique that often aids in the systematic design of experiments [31]. Box-Behnken design (BBD) was utilized to determine the

optimal combination of selected variables to conduct RSM analysis. This technique extensively analyses selected parameters and their interconnections [32]. The level of factors used in BBD are listed in Table 1. The design is based on three independent variables: volume of DES, volume of THF, and pH.

Table 1. Level of factors used in BBD.

Parameters	Unit	Lowest Value	Highest Value
Volume of DES	μL	30	500
Volume of THF	μL	200	900
pH		2	10

BBD serves as a valuable tool in minimizing the number of experiments, particularly during the initial stages. It facilitates the identification of influential factors in the response [33]. By conducting BBD (Box-Behnken Design), it is possible to identify crucial operational variables that can optimize the extraction efficiency of MCPA and 2,4-D herbicides. The variables were optimized using the two-level factorial design (low and high), as shown in Table 1. The effects of all variables on the extraction were determined through a series of 13 separate experiments. The present study utilized a statistical analysis technique known as analysis of variance (ANOVA) to investigate the impact of independent factors on microextraction efficiency. The results of ANOVA were determined by calculating the F and P-values, as shown in Table 2.

The determination of the significant association between the variables influencing the extraction efficiency, namely the volume of DES, volume of THF, and pH, and the response, represented by the peak area, was accomplished through the analysis of p-values and F-values. Models that exhibit greater F-values and lower p-values are suggestive of statistical significance. For a model term to be considered statistically significant, its p-value must be less than 0.05. Table 2 shows the F-values and p-values corresponding to the developed model, precisely 0.63 and 0.742 for MCPA, and 1.19 and 0.494 for 2,4-D herbicides, respectively. As shown, the main factors possess negligible effects, as indicated

by their high p-values (> 0.05). A significant lack of fit ($p < 0.000$) was observed in both models, showing that the models inadequately specify the relationship between the variables and the response. In addition, the goodness of fit of the model was assessed by examining the coefficient of determination (R^2), which were 0.6527 and 0.7814 for MCPA and 2,4-D, respectively. These data indicated that 65.27% and 78.14% of the variability in MCPA and 2,4-D, respectively. According to the R^2 value of the model, it is possible that the connection between the variables and the response is not entirely represented. This implies that additional optimization or an alternative method may be required to attain a more accurate fit with the data. A possible explanation for this phenomenon is the presence of a weak correlation between the variables, which may hinder the model's ability to forecast the accurate relationships among the variables [34]. Besides, this can be explained by the high p-value ($p > 0.05$), which suggests that none of the variables have statistical significance. Consequently, all the factors under consideration are independent, as they exhibit no mutual influence and do not significantly impact the interaction between one another. One of the variables can be changed independently without significantly impacting the response of the experiment. The following regression equation (1) and (2) represents the relationship between the variables studied (volume of DES, volume of THF, and pH) for MCPA and 2,4-D, respectively:

$$Y = 0.146 + 0.000148X_1 - 0.000090X_2 - 0.0077X_3 - 0.000001X_1^2 - 0.000000X_2^2 - 0.00024X_3^2 + 0.000000X_1X_2 + 0.000001X_1X_3 + 0.000011X_2X_3 \quad (1)$$

$$Y = 0.340 + 0.000748X_1 - 0.000800X_2 - 0.0235X_3 - 0.000001X_1^2 + 0.000001X_2^2 + 0.00055X_3^2 - 0.000000X_1X_2 + 0.000007X_1X_3 + 0.000022X_2X_3 \quad (2)$$

Where Y is absorbance, and X_1 , X_2 , X_3 are variables terms of volume of DES, volume of THF, and pH, respectively.

An interaction plot provides a visual representation of the relationship between a continuous response variable and the influence of a specific variable on another. This interaction plot shows the influence of two or more

variables on the response [35]. Figure 7 and Figure 8 illustrate the interaction effect of variables on the extraction of 2,4-D and MCPA extractions.

Table 2. Analysis of Variance (ANOVA) of MCPA and 2,4-D

Analyte: MCPA					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.007886	0.000876	0.63	0.742
Linear	3	0.004335	0.001445	1.03	0.490
vol of DES	1	0.001081	0.001081	0.77	0.444
vol of THF	1	0.000759	0.000759	0.54	0.515
pH	1	0.002496	0.002496	1.78	0.274
Square	3	0.002141	0.000714	0.51	0.703
vol of DES*vol of DES	1	0.001855	0.001855	1.33	0.333
vol of THF*vol of THF	1	0.000043	0.000043	0.03	0.873
pH*pH	1	0.000033	0.000033	0.02	0.887
2-Way Interaction	3	0.001410	0.000470	0.34	0.803
vol of DES*vol of THF	1	0.000475	0.000475	0.34	0.601
vol of DES*pH	1	0.000001	0.000001	0.00	0.980
vol of THF*pH	1	0.000933	0.000933	0.67	0.474
Error	3	0.004197	0.001399		
Total	12	0.012083			

Analyte: 2,4-D					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	0.064872	0.007208	1.19	0.494
Linear	3	0.029353	0.009784	1.62	0.351
Vol of DES	1	0.017391	0.017391	2.88	0.189
Vol of THF	1	0.011101	0.011101	1.84	0.268
pH	1	0.000861	0.000861	0.14	0.731
Square	3	0.026184	0.008728	1.44	0.385
Vol of DES*Vol of DES	1	0.003066	0.003066	0.51	0.528
Vol of THF*Vol of THF	1	0.012900	0.012900	2.13	0.240
pH*pH	1	0.000180	0.000180	0.03	0.874
2-Way Interaction	3	0.009335	0.003112	0.51	0.701
Vol of DES*Vol of THF	1	0.005184	0.005184	0.86	0.423
Vol of DES*pH	1	0.000182	0.000182	0.03	0.873
Vol of THF*pH	1	0.003969	0.003969	0.66	0.477
Error	3	0.018144	0.006048		
Total	12	0.083016			

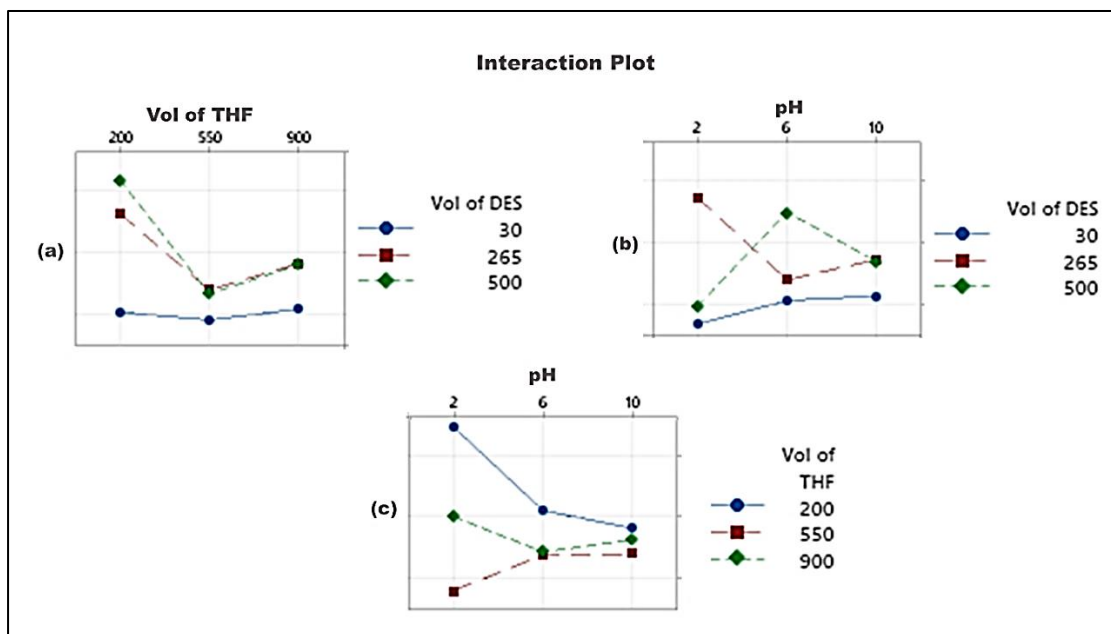


Figure 7. The interaction plot of 2,4-D sample, (a) volume of DES-volume of THF, (b) volume of DES-pH, (c) volume of THF-pH

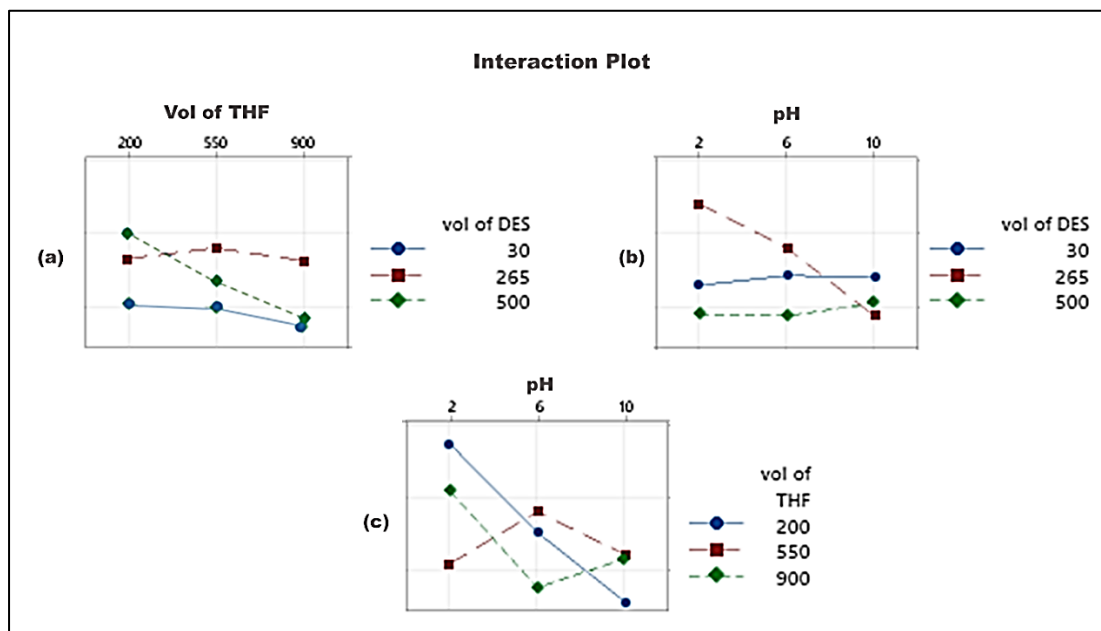


Figure 8. The interaction plot of MCPA sample, (a) volume of DES-volume of THF, (b) volume of DES-pH, (c) volume of THF-pH

As shown in Figure 7(a) and Figure 8(a), the absorbance exhibited a positive correlation with the increase in the DES volume and a decrease in the THF volume, specifically at a volume of 200 μ L. The enhancement of

mass transfer of 2,4-D and MCPA from aqueous phase into DES phase was observed when 500 μ L of DES was utilized. This improvement can be attributed to the establishment of sufficient hydrogen bonding and

hydrophobic interactions between the analytes and DES. The effective dispersion of DES in the aqueous phase requires the addition of an appropriate volume of THF, owing to the miscibility of DES in the aqueous phase. However, an increase in THF volume caused noticeable declines in response for both analytes. This can be attributed by the challenges encountered during emulsification of DES, which subsequently enhanced the solubility of the analytes in the aqueous phase. Consequently, this leads to a decrease in the extraction efficiency [26, 37].

Furthermore, it was observed that both 2,4-D (Figure 7(b) and 7(c)) and MCPA (Figure 8(b) and 8 (c)) exhibited good responses at acidic conditions with pH 2. The acidic condition enhances the efficacy of analyte extraction. This observation is consistent with the anticipated behavior of these herbicides, as they often possess acidic properties that aid in their solubility inside the hydrophobic DES [37]. However, with an increase in pH, there was a noticeable decline in extraction efficiency. This decline in extraction efficiency can be attributed primarily to the ionization of the herbicides during the reaction [9]. The ionization results in an increase in the herbicides' water solubility, posing a difficult in mass transfer into the hydrophobic DES phase. Consequently, the extraction efficiency diminishes as the herbicides' solubility in the aqueous phase increases. This study's findings indicate that the extraction level in acidic circumstances is similar to the outcomes observed in the experimental results (Figure 6). Thus, this observation further emphasizes the importance of considering the pH of the extraction system when designing and optimizing extraction methods for analytes in achieving high extraction efficiency.

Referring to the experimental result obtained above, there are some noticeable discrepancies between the experimental data and the predicted data from BBD, specifically volume of DES and THF for both analytes. These discrepancies between the experimental data and BBD suggested the potential existence of unaccounted factors or variables that could impact the effectiveness of the extraction process. This phenomenon arises from conducting experimental investigations by

systematically varying one variable at a time, whereas the other independent variables remained constant at their original levels. Consequently, the accuracy of this experiment may be compromised due to the challenge of ascertaining the relationship among all the variables [38]. This finding contradicts the BBD, which provided a simultaneous dual effect among the two variables. This feature helps the researchers in identifying the variables that make the most significant contribution to the model [39]. In order to improve the extraction, BBD suggested that high extraction efficiency can be achieved by utilizing a volume of 500 μL and 200 μL for DES and THF, respectively, for extraction of 2,4-D and MCPA.

The optimal condition determined through BBD involved 265 μL and 200 μL volume of DES and THF with pH 2 for both 2,4-D and MCPA. The optimal volume for DES was 265 μL , rather than the previously mentioned value of 500 μL . The rationale behind this decision was based on the potential diluting impact caused by an increase in DES volume, which subsequently reduces extraction efficiency. Furthermore, it has been proposed that the volume of THF should be similar to the volume of DES [9, 22]. Therefore, utilization of an equal amount of THF and DES results in better interaction and improved mass transfer of analytes to the DES phase from the aqueous solution [9].

The findings of this study demonstrate the advantages of utilizing BBD as a methodology for identifying the most favorable situations. Utilizing a BBD allows for a more complete and reliable extraction procedure by considering the combined impacts of various variables. This approach facilitates a comprehensive analysis of the relationships between variables and assists in determining the optimal circumstances for attaining a significant degree of extraction efficiency. Identifying ideal conditions provides valuable insights for future studies and can serve as a guiding principle to achieve high efficiency in similar extraction procedures.

Conclusion

In this study, a fatty acid deep eutectic solvent was successfully synthesized using lauric acid and pelargonic acid. The confirmation of DES was achieved

through FTIR spectra analysis, which enabled the identification of significant peaks associated with DES in the extraction process. The optimization of the variables influencing the ELLME method was carried out experimentally and theoretically. Furthermore, response surface methodology (RSM) based on Box Behnken design was utilized to optimize the overall extraction method, considering various factors and their interrelationships with each parameter. The findings of this study highlighted the significance of BBD as a suitable method for understanding and optimizing the complex relationship between multiple variables involved in the extraction process. The optimal conditions proposed through BBD for extraction of 2,4-D and MCPA can be achieved by applying 265 μL and 200 μL volume of DES and THF at pH 2. Thus, hydrophobic DES from a combination of lauric acid and pelargonic acid can functionally be used as an extraction solvent in the extraction process, especially for determination of pesticides. Studying BBD method provides valuable insights for future development in extraction method to be used on real sample since the extraction parameters were systematically evaluated, leading to improved extraction efficiency and accuracy.

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