Original Article

Modeling of the Pyridalyl Pesticide Extraction Process from the Aqueous Solution by Molecular Imprinted Polymer and Dispersive Liquid Liquid Micro Extraction

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Abstract

In this paper, we evaluate the performance of methods for extraction and separation of pyridalyl (pyl) pesticide from aqueous samples. In the solid phase extraction method, a non-covalently synthesized molecularly imprinted polymer is used. The properties of the synthesized polymers were investigated by BET and scanning electron microscopy. The effective parameters for pesticide extraction, such as adsorbent dose, pH, contact time and stirring rate, to achieve high removal percentage were studied using respond surface method. The capacity of solid phase (adsorbent) was also determined. In addition, DLLME method was used as a green, simple, rapid and sensitive method for measuring and removal of the pesticides. The effect of influencing factors on microextraction, such as type and volume ratio of dispersive and extractive solvents, salt concentration, pH and extraction time were investigated and optimized. The maximum recovery of the pesticide using SPE was obtained 75%. However, the recovery efficiency of 85% was achieved by DLLME. The results showed that both extraction methods have high potential to effectively reduce pesticides from aqueous solutions, however, the dispersive liquid liquid microextraction method has higher priority due to the very short separation time, higher transition aqueous phase to the organic phase, and the use of safe solvents.

Keywords: molecular imprinted polymer, dispersive liquid liquid microextraction, pyridalyl, pesticide removal, statistical modeling

INTRODUCTION

Molecularly imprinted polymers (MIPs) are similar in application and structure to antibodies, which have a specific function in identifying the target molecule [1]. The difference between molecularly imprinted polymers and antibodies is that antibodies have one or a limited number of locations to identify target molecules, while molecular imprinted polymers have several hundred to several thousand locations to identify target molecules. So, as a selection tool have many industrial, pharmaceutical and agricultural properties and applications [2]. Pyridalyl (somipleo) is a new and decisive insecticide to control the larvae of butterfly pests on tomato farms. Due to its special chemical structure and different effects from other toxins, this insecticide has a very good efficiency in controlling pests resistant to other toxins [3]. Figure 1 shows the molecular structure of pyridalyl.

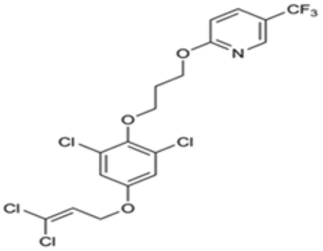

Figure 1: Pyridalyl molecular structure

Since pesticides are widely used in the food and agricultural industries and in various industries, and due to the high toxicity of these compounds, knowing their value is important even at low concentrations. Dispersive Liquid Liquid Micro Extraction (DLLME) method is proposed based on the generalization of the contact surface between the two liquid phases, which is a quick and easy extraction method. Liquid Liquid extraction and solid-phase extraction are among the widely accepted methods used to extract and pre-concentrate these compounds [4].therefore, in the present study, a simple and efficient method for extracting and pre-concentrating

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pyridalyl pesticide has been proposed by MIP and DLLME and process modeling is evaluated using experimental design method.

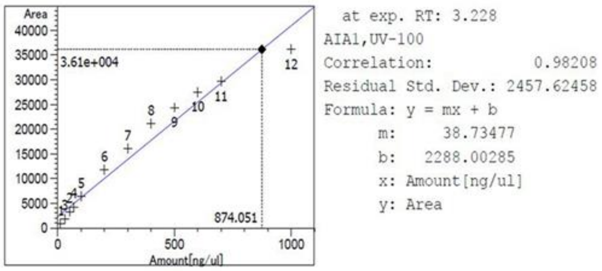
METHOD

Experimental Chemicals and Devices

Pyridalyl pesticide (98%) was obtained from Dr. Ehrenstorfer Chemicals (Augsburg, Germany). Molecularly Imprinted polymer (MIP) particles were prepared from Ethylene glycol dimethacrylate (EGDMA, purity: 98%) as a crosslinking agent and methacrylic acid (MAA, 98%) as a functional monomer, both purchased from Merck, Germany. In addition, 2, 2-azobis-2-methyl propionitrile (AIPN, 98 %, from Sigma-Aldrich Co) was used as an initiator. Phosphoric acid (65%, Merck, German) and sodium hydroxide (99%, Merck, German) was used to prepare phosphate buffer solutions as a carrier electrolyte. Organic solvents in this study were used for various applications; for example, as mobile phase in high performance liquid chromatography or dispersing solvent and extraction solvent such as: carbon tetrachloride, dichloromethane, chloroform, benzyl chloride, acetonitrile, methanol, ethanol, and acetone. All solvents were obtained from German Merck. The high Performance Liquid Chromatography, model Agilent 1290 Infinity II with four-solvent equipped with EX 1600 UV detector was used in study. This device contains a constant injection volume of 10 µL. The C- 18 non-polar column, with a diameter of 4.6 mm and a length of 250 mm, gives us the best separation with the machine conditions. The 827 metrohm pH meter was used to measure the pH of the solutions. The apparent structure of the MIP particles was studied using Mira 3-XMU field emission scanning electron microscope (TESCAN).

Determination of Pest by HPLC

Detection of Pyridalyl; Pesticide in samples was performed using HPLC. In order to draw the calibration curve, standard solutions of pesticides were made in the concentration range of 5-700 ng /µl; then these solutions were injected on the machine. The amount of Pyl was identified with UV detector at the wavelength of 285 nm as area under the plotted curve. The calibration curve of the pesticide was obtained by plotting linear diagram of area under curve in terms of pesticide concentration (Figure 2).



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  at exp. RT: 3.228
AIA1,UV-100
Correlation:             0.98208
Residual Std. Dev.: 2457.62458
Formula: y = mx + b
      m:      38.73477
      b:    2288.00285
      x: Amount[ng/ul]
      y: Area
```

Figure 2- Calibration Curve of Pyl Pesticide at 5-700 ng/µl.

Synthesis of MIP

Molecularly Imprinted Polymer particles for Pyl pesticide (Pyl-MIP) were synthesized through copolymerization of MAA and EGDMA. To this end, 0.3 mL MAA as monomer, 35.0 mL of dry chloroform as solvent and 0.2 g of Pyl as molecular template were combined and placed in a 100.0 mL round-bottomed flask, and the mixture was kept isolated for 15 min. Then, 20.0 mL of EGDMA as crosslinker and 0.22 g of AIBN as initiator were added to the mixture, the flask was then sealed, and the mixture was purged with N_2 gas for 15 min. The copolymerization was completed in a water bath at 65 °C within 24 h. For the purpose of removal of pesticide molecules from polymer structure, the resulting copolymer was dried and ground, and then washed at 10.0 % v/v acetic acid in methanol for 24 h. Therefore, no Pyl was detected in the solvent from the washing polymer by HPLC. In the next step, the synthesized polymers were washed three times with deionized water to remove the washing solution. The synthesized polymer particles were then dried in an oven at 55 ºC. Non-imprinted polymer (NIP) particles were also synthesized by a similar procedure, except that the MAA polymerization was performed in the absence of Pyl molecules.

Removal of Pyl by MIP

In order to obtain the highest rate of pesticide removal from aqueous by MIP based SPE, independent variables such as solution pH (2.0-10.0), MIP dose (0.1 -1.0 g), contact time of polymer particles with solution (10 - 75 min) and stirring rate of solution (400 - 800 rpm) were chosen and their effects on the removal of pesticide from the solution were investigated. Using MIP particles, the pesticide extraction conditions were optimized based on response surface statistical method, and a mathematical model was developed to predict pesticide removal from the solution. Indeed, with the help of this statistical method and with the least number of experiments, the simultaneous effect of all four independent variables and their interactive impact on pesticide removal was studied comprehensively. The design of experiments, statistical analysis of the obtained data and mathematical modeling were performed using Design of Expert V.11 software package [5]. By introducing the variables and their studied levels into the software (Appendix 1), 30 tests were proposed to optimize the conditions with the highest removal rate.

The microextraction of Pyridalyl pesticide by MIP-SPE was performed in optimum conditions as follows: 590 mg of MIP particles was added to 100 mL of 0.1 M phosphate buffer at pH 5.9 (as adsorption solution) containing 100 ppm pesticide and stirred for 58 minutes at 512 rpm on magnetic stirrer (Falcon 100), until a balance was created between the polymer and the pesticide solution. The resulting mixture was then centrifuged for 10 minutes at 5000 rpm (centrifuge device, Eppendorf, MiniSpin, Germany). The supernatant was prepared by passage of the solution in a 0.22 µm syringe filter for HPLC analysis. Concentration of residual pesticide in solution was obtained by HPLC equipped with UV-100 detector. Each solution was prepared and tested in three replications. The pesticide removal was calculated based on extraction recovery (ER %) using the following equation (1):

$$ER\ (\%) = \frac{C_0 - C_f}{C_0} \qquad (1)$$

Here, C_0 and C_f are the initial concentration and residual concentration of the Fen in the solution. In addition, the adsorption capacity of NIP and MIP particles for removal of Fen from aqueous was compared. In these tests, both polymers (NIP or MIP) were added to solutions containing pesticides and the separation process was carried out under optimum conditions. The polymer was then mixed with 3 mL of desorption solution (acetic acid / methanol) and stirred for one hour in the shaker at a speed of 200 rpm. The mixture was then centrifuged at 10,000 rpm for 10 minutes. The concentration of the pesticide in the supernatant solution (C_{ads}), which was the same as the amount absorbed by the polymer particles, was obtained by HPLC. The adsorption capacity of polymer particles (Q) (mg/g of polymer) was calculated using the following equation.

$$Q = \frac{C_{ads}V}{m} \qquad (2)$$

Where V is the volume of the solution (mL) and m is the amount of used polymer particles (g).

Removal of Pyl by DLLME

In order to obtain the best conditions for the removal of pesticide from the solution by using DLLME, with the highest removal efficiency, four operational factors including extraction solvent to dispersant solvent ratio (chloroform ratio to acetonitrile - 10 – 90 % v/v, namely; Sol ratio), extraction time (20 - 160 s), solution pH (3-11) and salt concentration (0.1 – 15.0 wt.%) were selected as independent variables affecting pesticide removal efficiency. Optimization of pesticide removal conditions was performed using response surface methodology and the design of experiment package software (V. 11). The studied variables and their range of study are listed in (Appendix 2). This software designs 30 tests based on the CCD method.

The extraction process was performed in the optimal conditions as follows: 0.1 mL of 100 ppm pesticide solution was added to 100 mL distilled water to reduce the standard sample concentration to 1 ppm. Then, 5.0 mL Fen pesticide (1 ppm) was transferred into the test conical tube, and 0.12 g of salt was added to it, and then the tube was shaken well until the mixture was completely dissolved. 300 µL mixture of extraction –dispersive solution (25/75) was injected to the solution containing Pyl, and the pH of the solution was adjusted to 6.0 with hydrochloric acid and ammonia. (The buffer solution cannot adjust the pH, as it may interfere with the ionic content in the solution and change the solution nature). The tube sealed and the solution was shaken for 72 seconds (extraction time) to form a cloudy solution. The tube was then centrifuged at 2000 rpm for five minutes. The solution was transformed into two phases in the lower phase, which is the organic phase, the solution was placed at the bottom of the tube, because of its heavy weight, the upper solution was separated by a syringe and injected directly into a HPLC to determine the amount of pesticide. The efficiency of DLLME method is characterized by an enrichment factor (EF) and Relative recovery (RR). Enrichment factor (EF) is calculated as shown in (3), where C_0 represents the initial concentration of the analyte in the sample and C_{sed} represents the concentration of the analyte in the sediment extraction solvent. The (RR) is also calculated according to (4), where C_{found} shows total amount of analyte found after addition of standard, C_{real} is the original concentration of analyte in the sample, and C_{add} is the amount of standard that was spiked into the original sample.

$$EF = \frac{C_{sed}}{C_0} \qquad (3)$$

$$RR = \frac{C_{found - }C_{real}}{C_{add}} \times 100 \qquad (4)$$

RESULTS and DISCUSSION

Study of FESEM images and BET table

The structure of the synthesized MIP for the solid phase extraction of Pyl Pesticide was observed by scanning electron microscopy and its morphologies were compared with non-imprinted polymer (NIP) particles. Figure 3 shows the FESEM image of the synthesized MIP and NIP polymer particles in this study, at 50,000 times magnification. As shown in the figure, the synthesized polymers have regular pores with approximately the same sizes. The presence of holes and pores in the MIP matrix is more regular and clearly visible, which is due to the removal of pesticide molecules from the polymer structure. The surface roughness on MIP particles is an advantage over the NIP, as the mass transfer of the Pyl molecules is performed easier on MIP, therefore the adsorption is increased. The specific surface area of the polymer was also checked with BET, According to Table 1, the molecularly imprinted polymer has a larger surface area and pore volume than the non-molecularly imprinted polymer That indicates there are more active absorption sites as well as more MIPs absorption capacity than NIP The diameter of the particles also indicates the Mesoporous structure in the polymer.

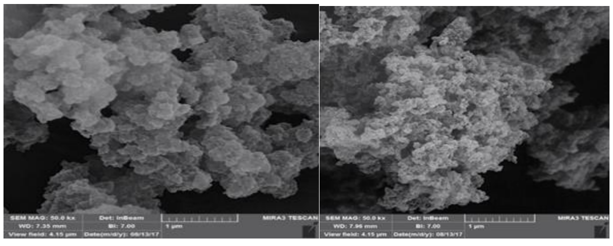


Figure 1: Figure 3 - FESEM Image of the Morphology NIP (left) and MIP (Right) Particles.

Table 1: BET analysis results

Brunauer, Emmett and Teller analysis of polymers			
Average pore diameter (nm)	Total pore volum ($cm^3.g^{-1}$)	Surface area ($m^2.g^{-1}$)	Polymer
10.4	0.52	196	MIP
12.6	0.30	95	NIP

Absorption Capacity of MIP and NIP

In order to evaluate the adsorption capacity of the synthesized polymer particles in pesticide removal, a series of solutions with a certain concentration of pesticide (100 - 500 ppm) were prepared. Then, 710 mg of MIP (or NIP) was added to 0.5 mL of pesticide solution with pH = 6.5 and stirred on magnetic stirrer for 58 minutes at 512 rpm. The polymer particles were separated from the solution by centrifuging and were placed in a desorption solution (1.0 % v.v acetic acid in methanol) for one hour. The mixture was then centrifuged at 10,000 rpm for 10 minutes. The concentration of pesticide in the supernatant solution was obtained by HPLC. Figure 4 shows the amounts of pesticide extracted from MIP and NIP particles versus different concentrations of Pyl. It is apparent in this figure that the amount of pesticide extracted in both polymers increases with increasing the initial pesticide concentration to 100 ppm and then reaches a constant value. It can also be seen that the extraction capacity of MIP and NIP is 54 and 2.0 mg/g, respectively. These indicate high adsorption capacity and high affinity of MIP particles to Fen pesticide compared to NIP particles. MIPs have a higher extraction rate due to the presence of molecular imprinted sites compared to NIPs. This result showed that the synthesis of MIP was performed well and that these polymers have the capability of selectively extracting the pesticide molecules.

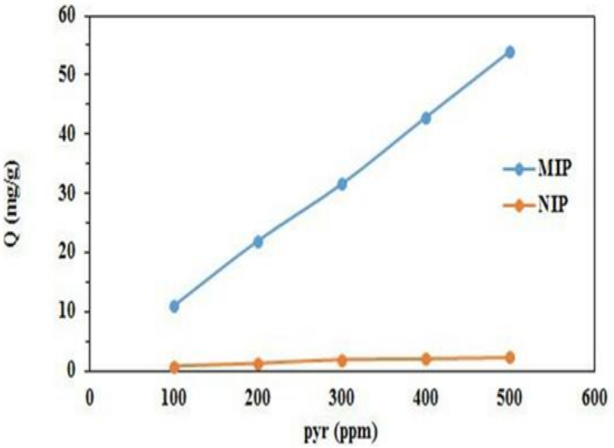


Figure 4: Comparison of the Adsorption Capacity of MIP and NIP for Removal of Pyl Pesticide

Optimization of extraction Conditions

Response Surface Methodology (RSM) is a set of statistical techniques used to optimize the processes in which the response is influenced by a number of variables. The graphical representation of the mathematical model defines the term of response surface method. The number of experiments is reduced with the help of this statistical design, and all the coefficients of the quadratic regression model and the interactions of the factors can be estimated. The most important issue of this research is to investigate the main effects and interactions of factors, hence the RSM scheme was chosen. In this study, RSM was used for both extraction methods for the separation and removal of Fen pesticide. In SPE, the effects of pH, contact time, amount of MIP particles and stirring rate as independent variables were investigated at five levels on extraction recovery. In DLLME, the effects of the ratio of the dispersive solution to extraction solution, salt concentration, pH and extraction time as independent variables were evaluated in five levels. In addition, the type of extraction solvent and the type of dispersive solvent were also investigated by single-factor method.

SPE with MIP

As previously mentioned, the central composite design (CCD) was used to design the RSM. The design of the experiment led to drawing the response surface curve and reaching the optimum point for SPE. In the CCD, the number of factors studied was four and the number of tests required was 4 + 26. Sixteen experiments were performed at axial points (2^4), eight tests at star points and six at center points. The least squares method was used to calculate model coefficients using Design Expert Software V. 11. Significance of regression coefficients was determined by F test at 95% confidence level. Analysis of variance (ANOVA) was performed to determine the significant quadratic models that fit the experimental responses and the independent variables. Model coefficients, F values and significant probabilities were considered. Statistical F values were used to check whether the regression models were appropriate for describing the observed data. Surface curves were used to show how a response variable depends on two factors based on the model.

The results of the ANOVA analysis are summarized in Appendix 3, the used model successfully predicted the responses. The P value represents the probable value used to determine the effect in a statistically significant model. The lower P represents higher significance. For statistical significance at 95% and 99% confidence levels, the P value should be less than or equal to 0.05 and 0.01 [5]. Fisher's statistical test was used to determine the significance of each factor, which is a significant degree based on the value of F ratio [6]. For this purpose, the F values of each parameter must be compared with the F values of the model. If the value of F is greater than the F value of model, the statistical test is significant at the selected confidence level.

Appendix 3 also shows that the linear coefficients A, B, C and D as well as factors interaction AB, AC, AD, BC and BD are all significant for extraction recovery of the pesticide.

Therefore, it is inferred that pH, the amount of MIP, time and speed of stirring, and the interaction pH - the amount of MIP, pH - time, pH - the rate of stirring, amount of MIP - time and amount of MIP- the rate of stirring have significant effects on the response. The significance of the presented model was evaluated by F and P values. The larger values of F and smaller of P mean that the applied model is more meaningful. The suggested F value for model of pesticide removal efficiency is 3.96. It can be seen that the obtained F values for the variables were much higher than the F values of the Fischer's model, which indicates that the model fits into the description of the SPE process by describing the pesticide extraction process.

Fig. 5 shows the surface response diagram for the interaction between pH and the amount of MIP particle on extraction recovery, when the micro-extraction time and stirring speed were 40 min and 600 rpm, respectively. The extraction recovery increased linearly with increasing pH in the range of 3.0 to 6.0 and decreased in alkaline conditions, namely, 6.0 to 10.0, respectively. In general, the relationship between extraction efficiency and pH can be related to the surface conditions and surface charge of MIP particles and pesticide molecules. In the molecular imprinting process, reversible bonds are formed between the functional monomers (herein methacrylic acid) and the template molecule (Pyl), which usually includes reversible covalent bonds, electrostatic interactions, cordinance with metal core or hydrophobic or van der Waals [7]. In this work, non-covalent bonds based on the hydrogen bonds between the template molecule (Pyl) and the functional monomers of MAA and EGDMA could easily be formed between the - OH groups and the C -O -, OH - and O - groups in the synthesis process of the MIP.

Given that the P_{Ka} of the MAA monomer is 4.65 [8], it can be expected that the functional groups of R-COOH in MAA will be deprotonated in mildly alkaline and acidic medium (>4.65) and converted to R-COO$^-$. However, Fen functional groups have positive partial charges, or at least neutral ones. It is therefore proposed that hydrogen bonds be formed between COO$^-$ and H bonded to O on Fen or COO-on Fen and -OH on MAA, thereby Fen being adsorbed on specific sites and accumulating on the surface of the MIP particles. It is also seen that increasing the amount of MIP particles to the optimum amount has a positive effect on extraction recovery. In this regard, it can be stated that the higher the MIP particles in the SPME, the higher the number of active and specific sites for the adsorption of pesticide molecules. As a result, more pesticide molecules are adsorbed onto the MIP particles and removed from the solution during the extraction process.

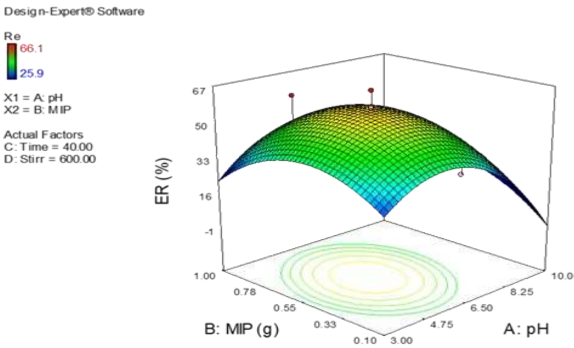


Figure 5: Three-dimensional Representation of Interaction Effect of MIP - pH on Pyl Extraction Recovery

Fig. 6 shows a 3D surface curve of the interaction between the time and the stirring rate of the solution on the extraction recovery, when the pH and the amount of MIP particles were 6.11 and 0.57 % wt, respectively. As can be seen from the figure, extraction recovery increased with increasing the contact time from 10 to 58 minutes and then reached a constant value. Contact time is one of the most important parameters in the adsorption and surface diffusion process [9]. Through studying the effect of contact time of MIP particles in the solution containing Fen, it can be stated that in 60 minutes, the pesticide molecules have sufficient time to be placed and permeate into the polymer matrix and occupy specific pores and saturate of polymer particles. As a result, the passage of time after the optimal time has no effect on the higher accumulation of pesticide molecules on MIP. On the other hand, it was observed that stirring speed in the range of 400 to 700 rpm has a positive effect on Fen extraction.

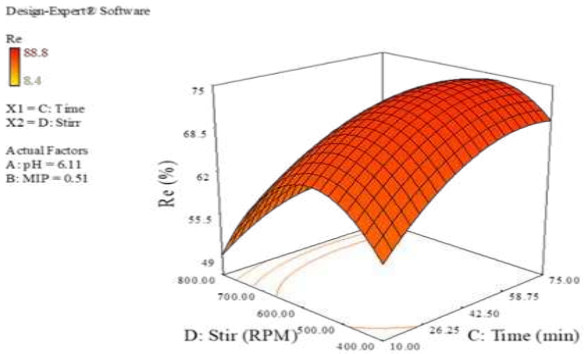


Figure 6: 3D Graph of Response Surface for Simultaneous Effect of Stirring Rate and Extraction Process Time on Pyl Extraction

One of the important goals of this study was to find the optimal operational parameters to increase the extraction recovery of pesticide, using mathematical model. The operating parameters was optimized based on a five-level CCD. In order to improve the process, a multi-response method called "the optimal function", which finds the operating conditions that have the best response, was used for this purpose. Table 2 shows the numerical optimization results for pesticide extraction.

Table 2: Optimal Values Obtained from Operational Variables and Response

pH	MIP (%wt)	Time (s)	Stirr (RPM)	Re (%)
6.5	0.71	60	620	%75

DLLME

The main advantage of this method is the fact that due to the rapid increase of the surface area between the extraction solvent and the aqueous sample and the rapid formation of cloud solution, a very fast equilibrium state is created, and as a result, the extraction time will be shorter. Therefore, in this study, it was tried to use the advantages of this method to remove Pyl pesticide from aqueous solutions. In this regard, the effect of parameters such as the type of extraction solvent, type of dispersive solvent, ratio of extraction solvent to dispersive, salt concentration, extraction time and pH on pesticide extraction was investigated.

Optimization of DLLME

Application of statistics in the design of experiments to investigate the various factors influencing responses reduces the number of experiments required to achieve optimal conditions and thus, reduces time and cost [10]. For this purpose, the central composite design was used to optimize the effective parameters in the micro extraction of the pesticide from aqueous solutions. Factors influencing this process include pH, extraction/dispersant solvent ratio, extraction time, and salt concentration. By introducing this range of variable levels into the Design of Expert software, as well as replication testing at the focal point, a total of 30 experiments were designed. Appendix 4 shows the random order of the experiments.

The results of analysis of variance (ANOVA) on pesticide extraction data by DLLME are summarized in Appendix 4. The model used based on the F value (1.58), obtained for pesticide removal efficiency and successfully predicted the responses. It is observed that the obtained F values for the variables are much greater than the F values of the Fischer model, thus indicating the compatibility of the model in describing the pesticide removal process by DLLME.Model compatibility was determined by correlation coefficient $R^2 = 0.9443$, indicating that changes in pesticide removal efficiency were more than 93% attributable to independent variables. In addition, the adjusted R value (Adj R = 0.923) close to the R^2 value indicates good agreement between the experimental and predicted responses. This indicates that the model used to predict the responses is valid. The coefficient of variation, obtained in this study (CV = 8.174%), indicates that the behavior of the experiments is correct and reliable.

The response variations in the studied ranges of the independent variables. In this section, the three-dimensional curves of the simultaneous effects of the independent variables, the extraction solvent ratio to dispersive solvent (10-90%), salt concentration (0.1 – 15 % wt), pH of solution (3-11) and microextraction time (20-160 seconds) on dependent variable, i.e. pesticide extraction efficiency, is shown in Figures 7 and 8.The changes of the Pyl extraction by DLLME as a function of the extraction solvent ratio to dispersive solvent and salt concentration are shown in Fig.7, at the extraction time and pH constant of 48 s and 5.0, respectively. As the graph shows, increasing the ratio of the extraction solvent to dispersive solvent from 10 to 30% v/v, had a positive effect on extraction efficiency, and excessive solvent ratio had an adverse effect on pesticide extraction. It was also stated above that the dispersive solvent will act as a bridge between the extraction solvent and the water, thereby causing the extraction solvent to be dispersed or converted into tiny droplets. On the other hand, higher amounts of dispersive solvent cause the dominating volume of dispersive solvent to volume of extraction solvent and the lower analyte to enter the organic phase and consequently, decrease the extraction efficiency.

It is also seen that salt concentration has a significant effect on extraction efficiency. Similar research has reported that the amount of ions in the aqueous sample has a great effect on phase separation and extraction rate. Since, some of the extraction solvent is dissolved in water, if the ions in the solution are high and the solution is made more polarized by adding ions, these ions can expel the extraction solvent, thus dissolving a small amount of the extraction solvent in water. Therefore, the remaining time of extraction solvent is reduced in the aqueous solution, and as a result, the extraction efficiency will be lower. Hence, the ionic strength of the solution should be such that the retention time of the solvent in the water is high, but the solution easily converts to two phases. Here, it was observed that the highest removal efficiency is obtained in salt concentration of 3.5 wt. %. This means that the salt in the range creates such an environment that extraction solvent is present for a long time in vicinity to the pesticide molecules.

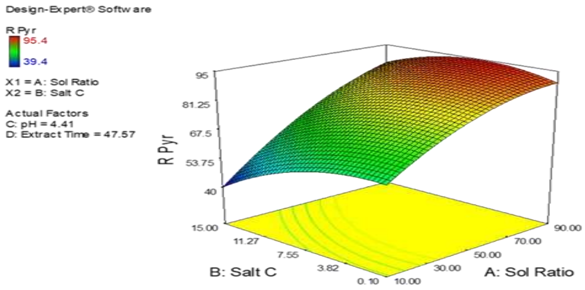


Figure 7: Changes in the Extraction Efficiency of Pyl Pesticide under the Influence of Extraction Solvent to Dispersive Ratio and Salt Concentration at Extraction time (48 s) and pH (5.0)

Figure 8 shows the dependence of changes in pesticide extraction by DLLME, under the influence of extraction time and pH at constant extraction solvent- dispersive ratio (35% vol.) and salt concentration (6.5 wt.%). It can be seen that the highest pesticide extraction was obtained under neutral conditions and the extraction efficiency decreased in mildly acidic and mild alkaline conditions. The pesticide molecules have several functional groups, turns into an acidic molecule because of its weak acidic strength in aqueous solutions. Thus, solution conditions must be such that the pesticide molecules are neutralized in the environment, so they dissolve more easily in organic solvents. In the neutral medium, the pesticide molecule does not have any acidic or alkaline agent, the so-called neutral molecule, so it is more easily dissolved in the extraction solvent and inserted in the organic phase and separated from the aqueous solution [11].

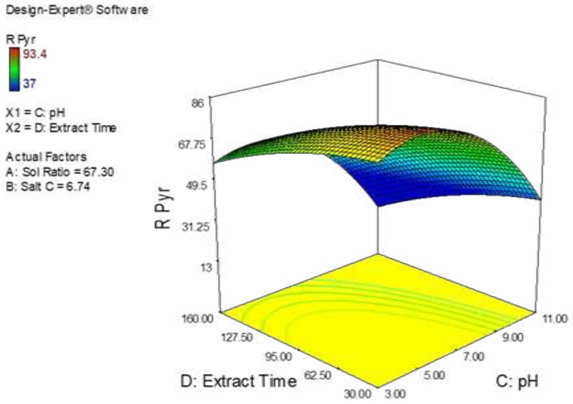


Figure 8: 3D Surface Curve of the Interaction of pH and Extraction Time on Extraction Recovery at Constant Extraction Solvent Ratio (67 % v.v) and Salt Concentration (6.7 wt %)

It is also seen in the curve that pesticide extraction efficiency improved by increasing the extraction time from 30 to 70 seconds, and longer extraction times had a negative effect on extraction efficiency. In the DLLME process, the extraction time is defined as the time between the injections of the solvents mixture until the centrifugation begins. At this time, organic solvents and water molecules interact with each other [12]. At low extraction times, the efficiency is less, because of the low interaction of the extraction solvent with aqueous solution. On the other hand, at times greater than the optimum time, emulsions of the extraction solvent in aqueous solution may be formed, which it makes difficult to separate the two phases. As a result, the remaining volume will be reduced.

The desirability function consisting of maximum pesticide separation efficiency by DLLME from aqueous was defined based on five level CCD plane. The developed model by CCD suggested the following conditions for effective reduction of the pesticide from solution.

Table 3: Optimal Conditions of Operational Variables of DLLME for Pesticide Extraction

Sol Ratio	Salt C (%wt)	pH	Extract Time (s)	ER
78/22	3.5	5.3	79	85

Real Sample

The efficacy of MIP based SPE and DLLME methods for removing Pyl pesticides from aqueous samples was investigated. In this experiment, the efficiency of extraction of Pyl from real water samples from agricultural wells was measured. The amount of toxins and organic compounds present in the real samples was calculated by determining the sub-peak surface of chromatograms of the sample and comparing it to the standard peak. Relative recovery was calculated as the ratio of analytical response in real samples and standard solution samples. If the pesticide peak was not detected in the samples, standard addition method was used, in which a certain amount of pesticide was injected into the samples. The results are presented in Appendix 5.

The data indicated that the Pyl pesticide was not detectable in the well water sample. The pesticide removal efficiency is generally acceptable by both micro extraction methods and these methods are capable of effectively separating the Pyl from aqueous solutions. These methods are able to reduce the amount of pesticide in the solution to the permissible limit and standard water values. The solid phase microextraction method was performed on the surface of synthesized MIP particles and Fen molecules were separated from aqueous through the interaction between the functional groups of the pesticide molecules and the polymer particles (methacrylic acid functional groups). On the other hand, it should be accepted that at very high pesticide concentrations, these sites are constant at the constant adsorbent mass (polymer particles), and the adsorption spaces will be occupied after the process is equilibrated; therefore, the separation efficiency will remain unchanged. This can be one of the drawbacks of the application of this method in high concentrations of organic compounds, especially pesticides.

Table 4- Results of Real Sample Analysis and Extraction Recovery by Micro Extraction Method

Sample	Pyl [ppm]				SPME	DLLME	
	Detected	Added	After microextraction				
			SP	DLL	ER (%)	ER (%)	EF
Well water	N.D	25	5	5.5	80	78	0.22
		50	10.3	10	78	80	0. 2

	75	24	21	77	79	0.21

CONCLUSION

The results indicated that both methods of the solid-phase extraction (SPE) using MIPs as well as dispersive liquid liquid microextraction (DLLME) have high selectivity in pesticide extraction from aqueous solutions. In the SPE, the MIP particles as the extraction phase have specific sites for the separation of the pesticide from aqueous solutions. The separation is, effective at low pesticide concentrations and seems to remain constant at high pesticide concentrations after the sites are occupied by Pyl molecules and the polymer surface is saturated. Unlike the solid phase method, in the DLLME method, since the structure of the method is different the separation process will be different, because in this method the transfer rate of aqueous and organic phases reaches its maximum value, so the highest extraction is achieved. Using this method, the removal process is done only once, and high efficiency is attained. Also, the time taken to prepare the sample without the deleterious effect on the sensitivity of the method is minimal. In addition, this method avoids excessive consumption of toxic organic solvents such as chlorine organic solvents. Consequently, dispersive liquid liquid microextraction is definitely recommended for pesticide removal, because of its rapidity, cheapness as well as low environmental damage.

Appendix

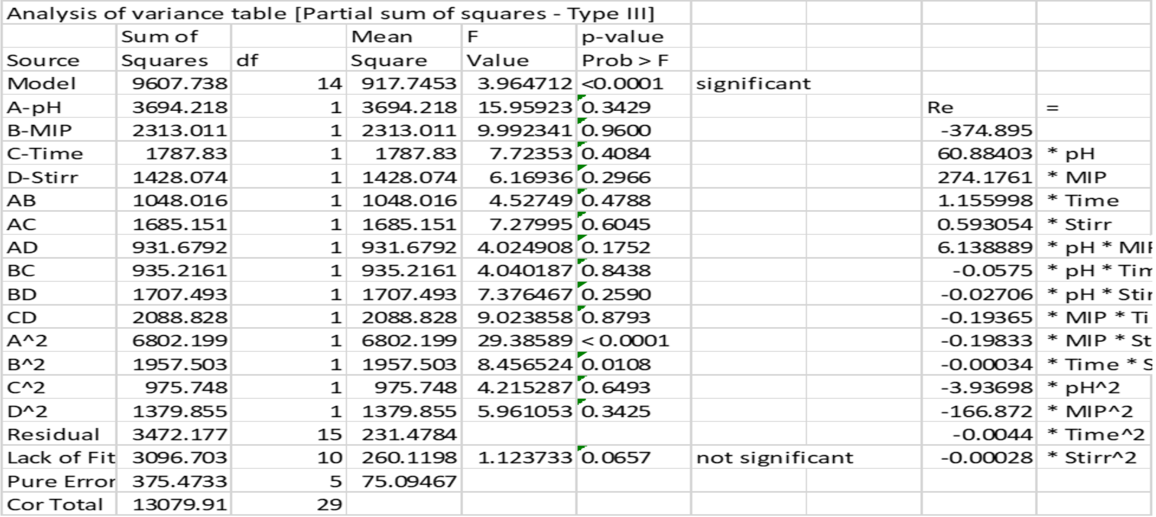
Appendix 1: Experimental range and levels of the independent variables

	Unit	Symbol	Levels				
			-α	-1	0	1	α
pH	-	A	2	4	6	8	10
MIP	g	B	0.1	0.33	0.55	0.75	1
Time	Min	C	10	26.25	42.50	58.75	75
Stirr S	rmp	D	400	500	600	700	800

Appendix 2: Experimental range and levels of the independent variables of DLLME

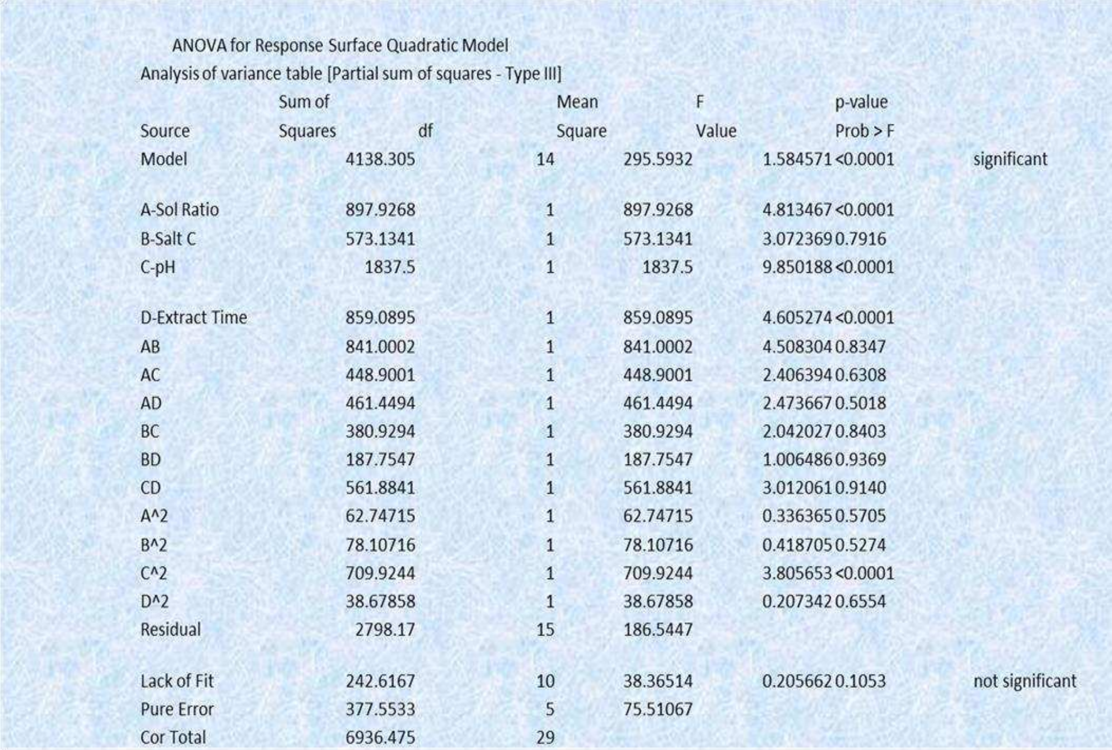
Parameters	Unit	Symbol	Levels				
			-α	-1	0	1	α
Sol Ratio	v/v	A	10	30	50	70	90
Salt	%wt	B	0.1	3.825	7.55	11.275	15
pH	-	C	3	5	7	9	11
Time	8	D	20	55	90	125	160

Appendix 3: The results of ANOVA for Model Developed from the Recovery Pyl by SPE



Analysis of variance table [Partial sum of squares - Type III]									
	Sum of		Mean	F	p-value				
Source	Squares	df	Square	Value	Prob > F				
Model	9607.738	14	917.7453	3.964712	<0.0001	significant			
A-pH	3694.218	1	3694.218	15.95923	0.3429			Re	=
B-MIP	2313.011	1	2313.011	9.992341	0.9600			-374.895	
C-Time	1787.83	1	1787.83	7.72353	0.4084			60.88403	* pH
D-Stirr	1428.074	1	1428.074	6.16936	0.2966			274.1761	* MIP
AB	1048.016	1	1048.016	4.52749	0.4788			1.155998	* Time
AC	1685.151	1	1685.151	7.27995	0.6045			0.593054	* Stirr
AD	931.6792	1	931.6792	4.024908	0.1752			6.138889	* pH * MI
BC	935.2161	1	935.2161	4.040187	0.8438			-0.0575	* pH * Ti
BD	1707.493	1	1707.493	7.376467	0.2590			-0.02706	* pH * Sti
CD	2088.828	1	2088.828	9.023858	0.8793			-0.19365	* MIP * Ti
A^2	6802.199	1	6802.199	29.38589	< 0.0001			-0.19833	* MIP * St
B^2	1957.503	1	1957.503	8.456524	0.0108			-0.00034	* Time * S
C^2	975.748	1	975.748	4.215287	0.6493			-3.93698	* pH^2
D^2	1379.855	1	1379.855	5.961053	0.3425			-166.872	* MIP^2
Residual	3472.177	15	231.4784					-0.0044	* Time^2
Lack of Fit	3096.703	10	260.1198	1.123733	0.0657	not significant		-0.00028	* Stirr^2
Pure Error	375.4733	5	75.09467						
Cor Total	13079.91	29							

Appendix 4: Results of Analysis of Variance on the Proposed Model for Pesticide Extraction by DLLME



ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4138.305	14	295.5932	1.584571	<0.0001	significant
A-Sol Ratio	897.9268	1	897.9268	4.813467	<0.0001	
B-Salt C	573.1341	1	573.1341	3.072369	0.7916	
C-pH	1837.5	1	1837.5	9.850188	<0.0001	
D-Extract Time	859.0895	1	859.0895	4.605274	<0.0001	
AB	841.0002	1	841.0002	4.508304	0.8347	
AC	448.9001	1	448.9001	2.406394	0.6308	
AD	461.4494	1	461.4494	2.473667	0.5018	
BC	380.9294	1	380.9294	2.042027	0.8403	
BD	187.7547	1	187.7547	1.006486	0.9369	
CD	561.8841	1	561.8841	3.012061	0.9140	
A^2	62.74715	1	62.74715	0.336365	0.5705	
B^2	78.10716	1	78.10716	0.418705	0.5274	
C^2	709.9244	1	709.9244	3.805653	<0.0001	
D^2	38.67858	1	38.67858	0.207342	0.6554	
Residual	2798.17	15	186.5447			
Lack of Fit	242.6167	10	38.36514	0.205662	0.1053	not significant
Pure Error	377.5533	5	75.51067			
Cor Total	6936.475	29				

REFERENCES

1. Holcomb, L.C.; Seabrook, B.S.; Indoor Environ. 4, 7-26, 1995.
2. Medeiros-Vinci, R.; Jacxsens, L.; Van Loco, J.; Chemosphere. 88, 1001-1006, 2012.
3. Poucke, C.V.; Detavernier, C.; J. Agric. Food Chem., 56, 4504–4510, 2008.
4. Mariana Lepri de Oliveira, Bruno Alves Rocha, Vanessa Cristina de Oliveira Souza, Fernando Barbosa, Determination of 17 potential endocrine-disrupting chemicals in human saliva by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry, Talanta, 196, 2019, 271-276, https://doi.org/10.1016/j.talanta.2018.12.067.
5. Jafari, S., Dehghani, M., Nasirizadeh, N. and Baghersad, M. H. (2019), Synthesis and characterisation of a selective adsorbent based on the molecularly imprinted polymer for the removal of cloxacillin antibiotic residue from milk. Int J Dairy Technol, 72: 505-514. doi:10.1111/1471-0307.12620
6. Ali Nazari, Mohammad Mirjalili, Navid Nasirizadeh, Shahab Torabian, Optimization of nano TiO2 pretreatment on free acid dyeing of wool using central composite design, Journal of Industrial and Engineering Chemistry, 21, 2015, 1068-1076, https://doi.org/10.1016/j.jiec.2014.05.017.
7. Tzeng, Chorng-Jyh, & Rui-Yang Chen."Optimization of electric discharge machining process using the response surface methodology & genetic algorithm approach. "Int. J. of precision engineering & manufacturing 14.5 (2013): 709-717.
8. N. T. Abdel-Ghani, A. K. Hegazy, G. A. El-Chaghaby and E. C. Lima, "Factorial experimental design for biosorption of iron and zinc using Typha domingensis phytomass," *Desalination,* 249, 2009, pp. 343-347.
9. Tayebeh Esfandiyari, Navid Nasirizadeh, Mohammad Hassan Ehrampoosh, Masoumeh Tabatabaee, Characterization and absorption studies of cationic dye on multi walled carbon nanotube–carbon ceramic composite, Journal of Industrial and Engineering Chemistry, 46, 2017, 35-43, https://doi.org/10.1016/j.jiec.2016.09.031.
10. Justyna Płotka-Wasylka, Małgorzata Rutkowska, Katarzyna Owczarek, Marek Tobiszewski, Jacek Namieśnik, Extraction with environmentally friendly solvents, TrAC Trends in Analytical Chemistry, 91, 2017, 12-25, https://doi.org/10.1016/j.trac.2017.03.006
11. Nour Al-Afy, Hassan Sereshti, Akram Hijazi, Hamid Rashidi Nodeh,Determination of three tetracyclines in bovine milk using magnetic solid phase extraction in tandem with dispersive liquid-liquid microextraction coupled with HPLC, Journal of Chromatography B, 1092, 2018, 480-488, https://doi.org/10.1016/j.jchromb.2018.06.049.
12. Ksenia Cherkashina, Christina Vakh, Sofya Lebedinets, Aleksei Pochivalov, Leonid Moskvin, Aleksey Lezov, Andrey Bulatov, An automated salting-out assisted liquid-liquid microextraction approach using 1-octylamine: On-line separation of tetracycline in urine samples followed by HPLC-UV determination, Talanta, 184, 2018, 122-127, https://doi.org/10.1016/j.talanta.2018.02.112.