Development of a Solid - Phase System for the Extraction of Diazinon from Water

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Authors' contributions

This work was carried out in collaboration among all authors. Author JDN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RS and JOI managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

A selected sorbent granulated activated charcoal solid - phase extraction was developed and used to extract diazinon from distilled water (TDW), distilled deionized water (DDW) and stream water (DW) was studied in the laboratory. Prior to the extraction, the half-live of the diazinon was determined in buffer solutions of pH 6, 7 and 8. The study showed that the half-lives were found to be 0.074, 0.022 and 0.077 hours respectively. Aqueous solution of diazinon was extracted in triplicate from distilled water, deionized distilled water and stream water using SPE systems. The extracted analyte was analysed by uv-visible spectrophotometer. The mean percentage recovery from distilled water, deionized distilled water and stream water using SPE 88.58%, 78.74% ad was 67.85% respectively while diazinon recovery using Liquid-liquid Extraction techniques gave a mean percent recovery of 96.60%, 93.80% and 75.54% for DW, DDW ad SW respectively. The mean percent analyte loss for SPE systems was 32.79%. The value was approximately twice higher than those obtained from LLE techniques with a value of 14.16%. The mean percentage recovery and analyte loss for SPE and LLE were compared. Similarly, the second order rate constant of diazinon of pH6, 7 and 8 were 2.6×10^{-3}, 8.8×10^{-3} ad 2.5×10^{-3} $M^{-1}S^{-1}$ respectively. The study suggests that granulated activated charcoal can be used as an sorbent to extract diazinon from water.

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1. INTRODUCTION

Organophosphorus insecticides are class of pesticides that are esters or organic salts of phosphoric acid or its derivatives, which are used to kill insects that are detrimental to man in agricultural and non-agricultural activities. Generally, these compounds are highly toxic to target organisms as well as mammals although some are less toxic to mammals [1]. Once an organophosphorus insecticide is introduced into the environment (biota, soil, water, air) can be translocated, transformed or may persist due to factors such as photolysis, volatility and sorption processes [2]. The transport of pesticides out of their area of application results in the presence and subsequent accumulation of these compounds or their degradation products in many parts of the environment including water. However, in addition to definite advantages for the food production efficiency, these pesticides also have an increasing negative impact on the aquatic lives and also affect human beings that depend solely on such stream or river for drinking and other domestic activities [3].

To monitor water pollution by pesticides, precise information on their concentration levels (trace and ultra-trace) in the environment is necessary. For this purpose, conventional methods are used for their analysis [4]. Solid Phase Extraction (SPE) technique is an alternative to classical analytical extraction methods used to separate analyte from liquid matrices. SPE require less organic solvents than the traditional extraction methods. Due to high purchase and disposal costs of many organic solvents, the SPE has resulted in substantial savings and environmental protection. SPE is a higher technique replacing the conventional Liquid-Liquid Extraction method most commonly performed using the separating funnel [5]. Several studies have been publish about the use of SPE technique to extract organic compounds from water using adsorbents such as C_{18} and XAD-2 resins as the stationary phase but there have been little literature on the use of granulated activated charcoal as an adsorbent to extract organophosphorus insecticides from water. This paper presents the development of a Solid-Phase Extraction system using granulated activated charcoal (GAC) to extract diazinon from water. The objectives are to carry out a laboratory study on the efficacy of using solid-phase extraction systems using granulated activated charcoal to extract diazinon from water.

2. MATERIALS AND METHODS

2.1 Sampling of stream water

Prior to sampling, the glass bottles used for collection of stream water were washed with detergent and rinsed with DW and acetone. The stream water was collected from one point in Agu stream in Daudu (latitude 7° 44′ N and longitude 8° 31′E) Lafia-Makurdi road in Guma Local Government Area of Benue State, Nigeria with a glass bottle and covered with screw-top Teflon to avoid contamination during transport and storage in the laboratory for physicochemical determination and extraction studies. The choice of the stream is because it's environment has been mainly used for farming activities especially cultivation of cassava, yams, millet, corn and sesame-seeds. Hence, organophosphorus insecticides may by applied on the farm for preservation which can be migrated to the stream. Plastic containers were not used because polyethene contains traces of plasticizers which may leach from the plastic to water or organics from water may adsorbed onto the plastic. Some physicochemical properties of the water sample are presented in Table 1. 10L each of distilled water and DDW was bought from Benue State Environmental Protection Agency Laboratory, Makurdi. The water samples were maintained at room temperature during transportation and storage in the laboratory.

2.2 Chemicals

All the chemicals used were analytical grade reagents (AnalaR) except commercial diazinon bought from Novatis crop protection AG Basel Switzerland. The pesticide was selected because it is widely used in Benue State for preservation of grains and is likely to pose residue problems in environmental media, including water. The pesticide was used directly as provided by the manufacturer without further purification. This is also in the form they are normally applied by the farmers on their farms and grains. The trade names of this product include Basudin, Dazzel, Gardentox Kayazol, Cobra and spectracide. However the IUPAC name is o, o-diethyl-o-2-isopropyl-6-methyl pyrimidin-4-yl Phosphorothio-ate but henceforth shall be called diazinon.
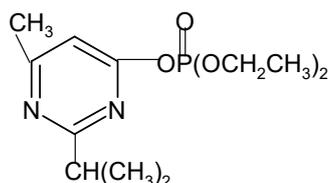
Structure of diazinon

Diazinon may be found in formulations with a variety of other pesticides such as pyretherins, lindane(1,2,3,4,5,6-hexachlorocyclohexane) disulfton. It is used on home gardens on a variety of sucking and leaf eating insects. It is also used on rice, sugar cane and on horticultural plants. It is available in dust, granules, wettable power and emulsifiable concentrate. The toxic effects of diazinon are due to the inhibition of acetyl cholinesterase, an enzyme needed for system function. Symptoms which are associated with diazinon poisoning in humans includes weakness, headache impaired vision, salivation, sweating nausea, vomiting, diarrhea and abdominal pain. Death has been reported for some instances from both dermal and oral exposures [6]. Physical properties. Molecular Formula: $C_{12}H_{21}N_2O_3PS$. Molecular Weight: 304.4. Colour: dark-brown liquid. Boiling point: 83-84°C /0.0002mmHg. Specific gravity: 1.116-1.118. Solubility: water (40ppm)/20c, ethanol, xylene, acetone, petroleum. The diazinon ($1.97 \times 10^{-7}M$) stock solution was prepared in distilled water by diluting $0.10cm^3$ of the stock diazinon, using 1uL graduated Hamilton gas-tight syringe, in a 1L volumetric flask, stopper with a glass stopper. The preparation of stock solution was done by methods described by Guenzi [2].

2.3 Methods

2.3.1 The degradation of diazinon in buffer solutions

The degradation of organophosphorus insecticides in the environment including water [7] is one of the most important factors in evaluating their fate in water as well as their possible adverse effect [8]. Thus, the ability to measure or reliably estimate the possible degradation process or pathway is of critical importance to the environmental risk assessment of these chemical [9]. Diazinon degradation in buffer solutions was measured using the following procedure:

Approximately $2.0cm^3$ of buffer solution of pH 6 was injected into the silica glass cuvette and placed in the compartment of the uv-visible spectrophotometer for 10mins in order to equilibrate the solution. The reaction was initiated by the addition of $0.5cm^3$ of diazinon ($1.97 \times 10^{-7}M$) using Hamilton gas-tight syringe. The final volume in the cuvette was $2.5cm^3$ and a wavelength of 247nm was set on the UV-Visible Spectrophotometer. The absorbances were manually measured at five minutes interval that lasted for 60 minutes and each absorbance was measured in duplicates. The measured absorbance was used in pseudo-first order plot using B+ log ($A_t - A_i$) on the vertical y-axis and time on the horizontal x-axis using a computer, Rate constants (K_{obs}) = - 2.303 x slope, Rate constants (K_{obs}) V_s buffer solution plotted to obtain second order rate constants and the half-lives ($t_{1/2}$) of diazinon was calculated using $t_{1/2}$ = ln2/k. where $t_{1/2}$ is the half- life of diazinon and K = rate constant obtained from the second order plot.

2.3.2 Affinity assessment of GAC by solid phase extraction

First an attempt was made to assess whether GAC could be utilized as a sorbent for loading. The granulated charcoal was activated at 50°C in an oven for 24 hours and cooled in a desiccator before use. After the activation process, an empty column of size 23cm long with an internal diameter of 1.73cm was loaded with polyethylene frits at the bottom followed by 1.0g of granulated activated charcoal and finally loaded with polyethylene frits at the top of the SPE column to avoid the sorbent becoming segregated. This gave the column a load of bed height of 5.5cm. The loaded SPE column and the separating funnel were connected and clamped on a stand while the collection vessel was connected to a vacuum pump and the SPE column.

To assess the affinity of GAC in aqueous solution, after conditioning the GAC- SPE column with $10cm^3$ methanol followed by $10cm^3$ n-hexane and finally with 10mL of distilled water to promote wetting and uniform flow through it [10]. The solution was quantitatively transferred into a separating funnel and its tap opened and allowed to pass through the loaded SPE column at a flow rate of approximately $3cm^3$/min using the vacuum pump without allowing it to get dry. Separation efficiency was maximized by having a continuous partitioning of analyte on the granulated charcoal as the spiked sample pass through the loaded column. After, all the diazinon solution in the separating funnel was emptied

through the loaded SPE column was washed with 5cm^3 of DW follow by elution with 10cm^3 of n-hexane introduced into the column and allowed to soak for 15 minutes to dissolve the analyte that have been adsorbed onto the sorbent before elution with another 10cm^3 hexane. The extracts were collected and concentrated in a rotator evaporator before quantification in the UV-Visible Spectrophotometer and the percentage recovery calculated. The extractions were carried out in triplicates. The same procedure was used for SW and DDW.

2.3.3 Quantitative recovery study using LLE

Approximately 2.0 cm^3 of aqueous solution of diazinon (3.94 X 10^{-3}M) was added to a glass silica cuvette, P and was kept in cell 1 of Jenway 6305 UV-visible Spectrophotometer. Then distilled water was added in the reference cuvette, R and kept in the reference compartment of the spectrophotometer and the wavelength was set at 247nm to monitor the disappearance of the diazinon in the solution and the absorbance was measured.

10 cm^3 of diazinon (3.94 x 10^{-3}M) was added to 20cm^3 of distilled water separately resulting in the final concentration to be 1.97 x 10^{-3}M. The solution was homogenized and transferred into a 250cm^3 separating funnel. Then 3.0cm^3 of n-hexane was added and shaken intermittently and vented out after 5 minutes. The separating funnel was clamped to allow the phases to separate

out. After 15 minutes. The organic phase was extracted into a vial and then capped. Three successive batch extractions were carried out so that all the analytes were removed from the distilled water by n-hexane. The extracts were pooled into a vial and concentrated to a volume of 3cm^3 by a rotary evaporator. The concentrate was measured at a wavelength of 247nm and the percentage recovery calculated.

Percentage recovery from water samples =

$$\frac{\text{Absorbance after extraction}}{\text{Absorbance before extraction}} \, x \, 100$$

3. RESULTS AND DISCUSSION

3.1 Wavelength Scanning Method

In this study, a wavelength scanning method over 200-500nm was carried out for diazinon in distilled water was measured at room temperature and a maximum wavelength of 247nm was chosen as shown on Fig. 1 to monitor the disappearance of diazinon in distilled water. The chosen maximum wavelength value was compared with the literature value of 245nm [11]. The difference in the wavelength maximum of this study and that of the literature value may be as a result of additives or impurities in the diazinon since it was not purified.
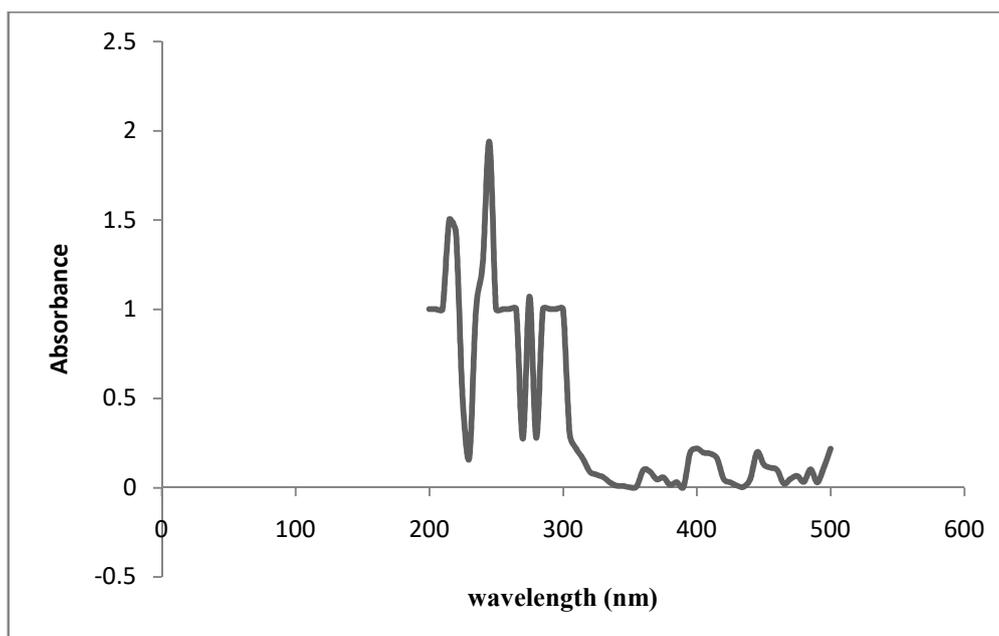

**Fig. 1. Wavelength scan of diazinon from 200-500nm**

3.2 The kinetic studies

Prior to extraction, although the samples were stored in the dark at room temperature, it was necessary to carry out a kinetic study using UV-Visible Spectrophotometer to establish if diazinon would remain intact for long enough in water to be extracted. A graph of mean absorb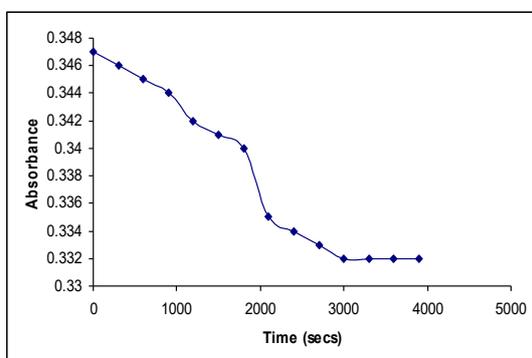ance against time for the degradation of diazinon in buffer solution of pH 6-8 shown on Fig. 2. This lead to pseudo-first order plots on Fig. 3. The second order rate constant K_{obs} was obtained by plotting the rate constant K_{obs} against buffer solution of pH 6-8 (Fig. 4). The second order rate constant K_2 (Table 1) was obtained from the slope and was used to calculate the half-live of diazinon in various buffer solutions.
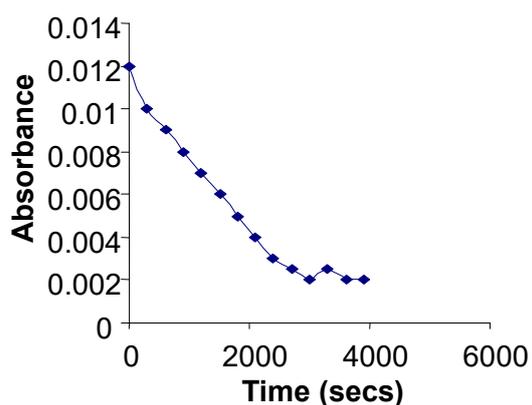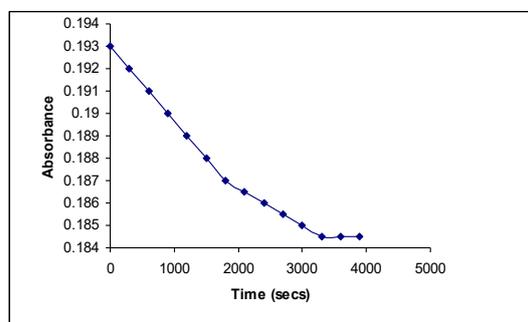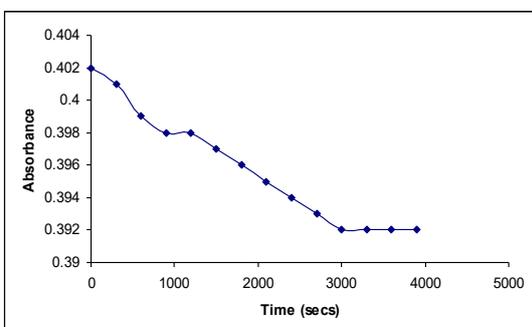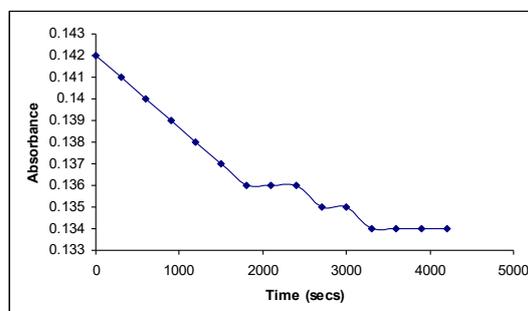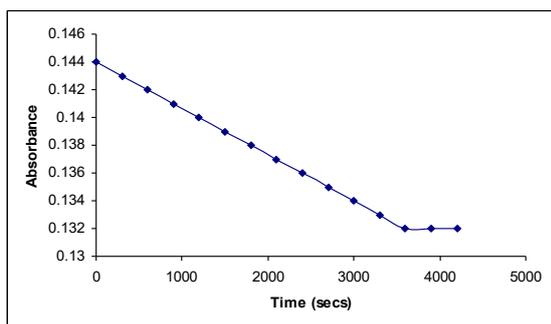

pH 7.89
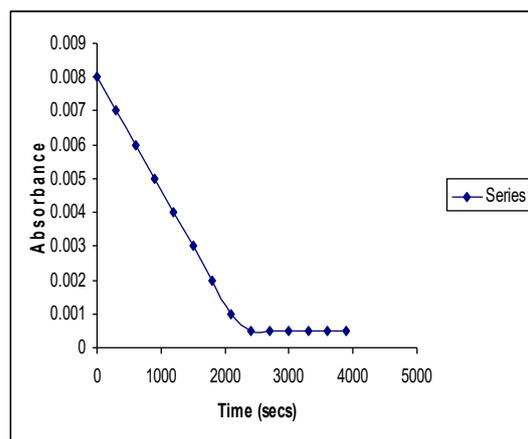
pH 7.48

pH 7.30

pH 6.87

pH 6.65

pH 6.43

pH 6.36
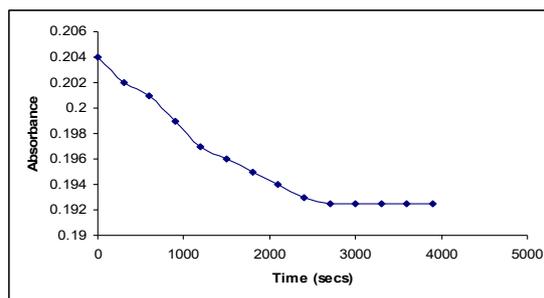
pH 5.87
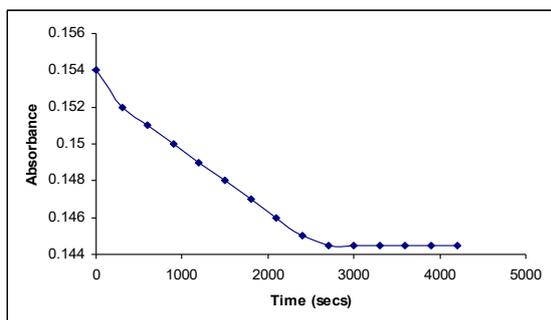
pH 5.65

pH 5.65

Fig. 2. A typical plot of absorbance versus time for the degradation of Diazinon (1.97x10^{-7}M) in buffer solutions at 247nm
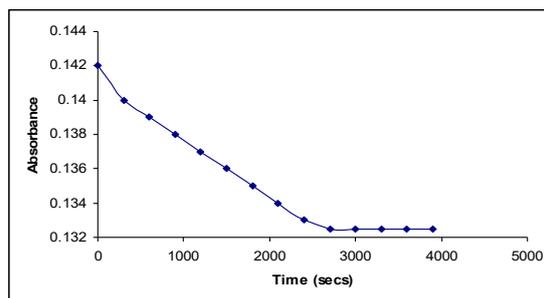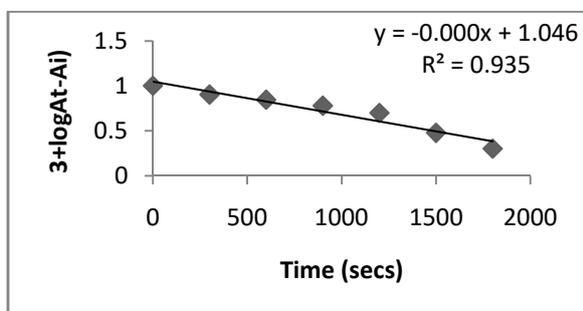

$y = -0.000x + 1.046$
$R^2 = 0.935$

$y = -0.000x + 0.966$
$R^2 = 0.927$

pH 7.89

pH 7.73

$y = -0.000x + 1.213$
$R^2 = 0.995$

$y = -0.000x + 0.981$
$R^2 = 0.971$
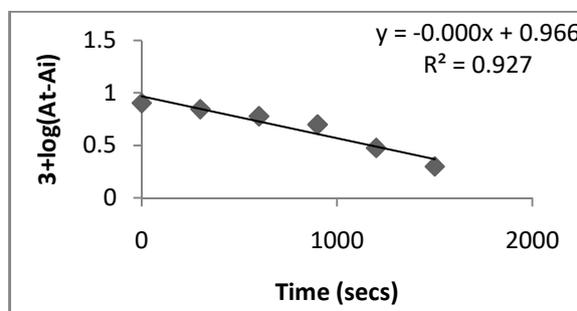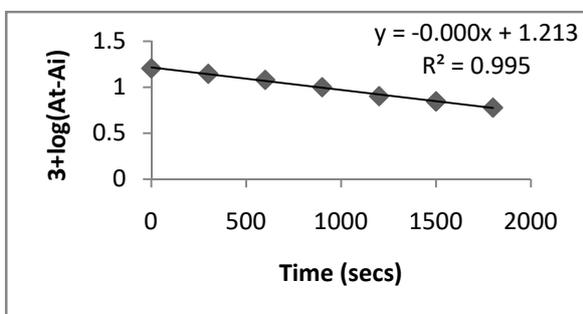
pH 7.45
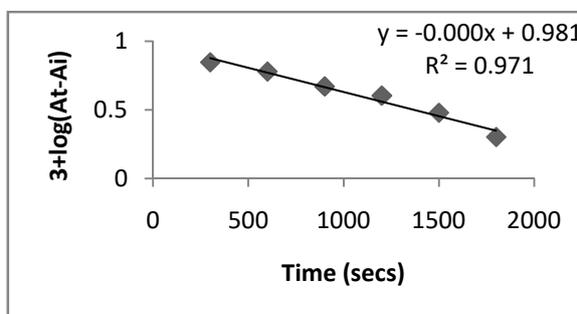
pH 7.30

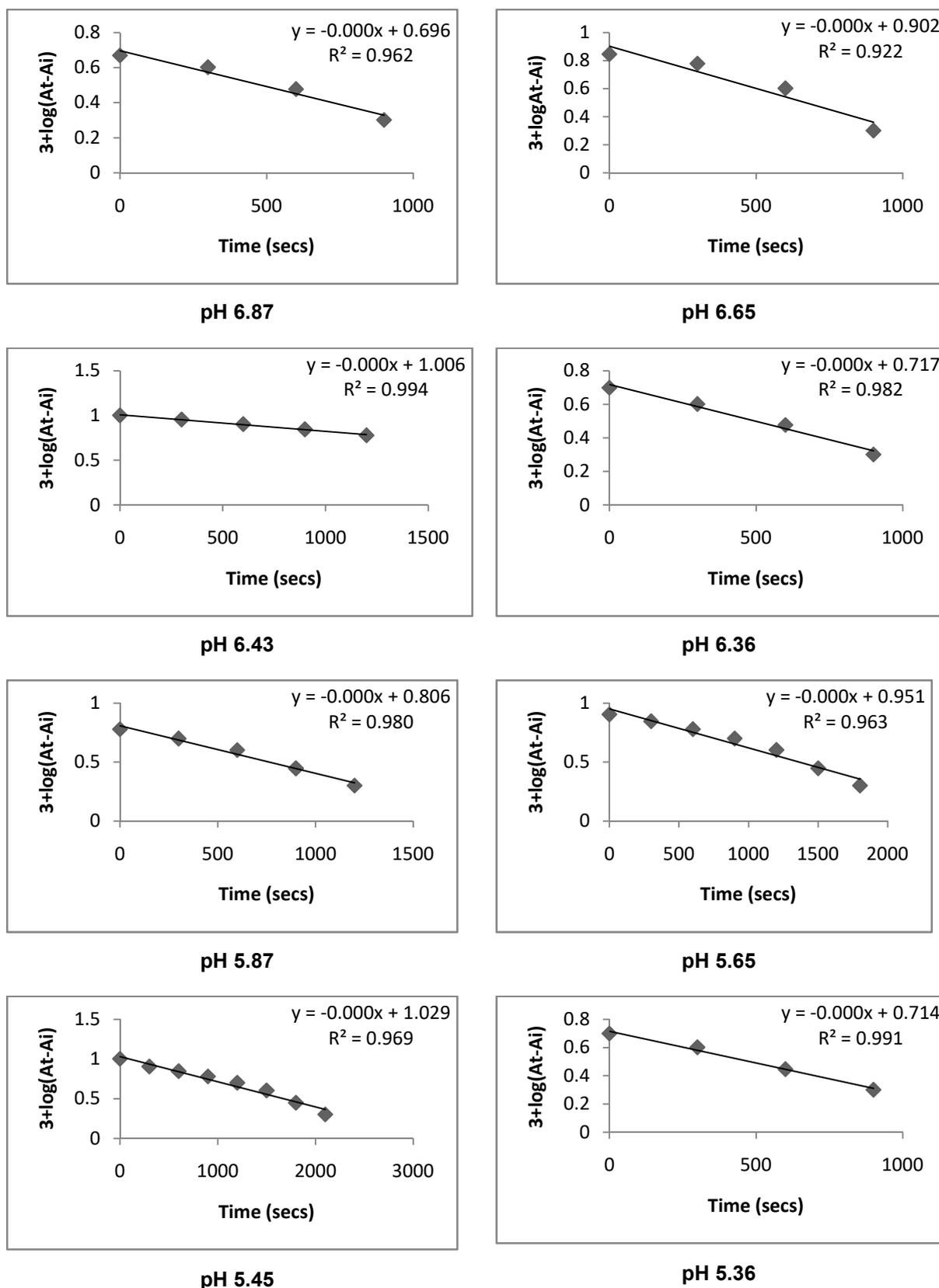**Fig. 3. Pseudo-first order plot for the degradation of Diazinon (1.97x10-7M) in buffer solutions at 247 nm**
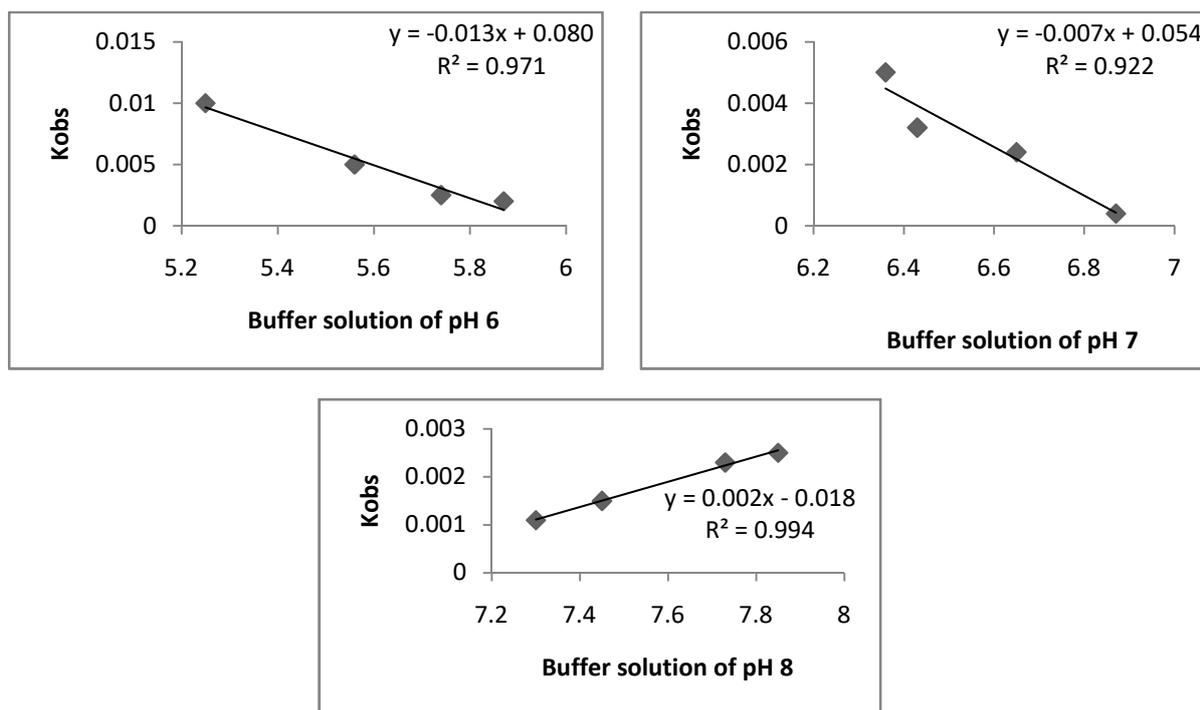
Fig. 4. A Second-order plot for the degradation of Diazinon in buffer solutions

Table 1. Second order rate constants of diazinon in buffer solutions

pH	Rate constants(K)M^{1}S^1
6	0.0026
7	0.0088
8	0.0025

Table 2. Some properties of stream water

S/No	Parameters	Quality
1.	Temperature	27°C
2.	pH	6.95
3.	Total hardness	17.50mg/L of CaCO$_3$
4.	Total alkalinity	252.3mg/L as CaCO$_3$
5.	Appearance	Dull
6.	Taste and odour	Unobjectionable

3.3 Properties of Stream Water

Some important physicochemical properties of stream water are presented in Table 2.The stream water was slightly coloured with a pH 6.75 indicating that it is slightly acidic (there are more hydrogen ion concentration than hydroxyl ion in the water). This may be due probably to the presence of humic or fulvic acids that have been formed in the water from the decomposition of vegetation or organic and inorganic substances dissolved in it. The result obtained from some properties of SW shows the presence of some of the specific matrices that are likely to interact with the pesticides during extraction. The distilled water (DW) and deionized distilled water (DDW) have pH 7.02 and pH 7.10 respectively indicating they are approximately neutral indicating that there are approximately equal numbers of hydrogen ion and hydroxyl ion concentration.

3.4 The Half – Lives

The half - live of diazinon in buffer solutions of pH 6-8 were calculated using the formula $t_{1/2} = \ln 2/K$. Where $t_{1/2}$ is the half life of the diazinon, K is the rate constant obtained from the second order plot as shown on Appendix 3. The values of the half - live of diazinon obtained in this study (Table 3) increases as the solution become either more acidic or more basic, relative to the rate observed at neutral pH. This is in agreement with the studies carried out by Luther, et al. [12] that, the degradation of pesticides exhibit marked pH dependence. Also, Eneji [13] and Momoh et al. [14] reported generally that, their degradation decreases in buffer solution as the solution gets closer to pH 7 and increases in mildly acidic and basic medium. Their half - lives shows that both

pesticides will degrade slowly in the buffer solutions and can remain intact long enough in the solution to be extracted from water within the period of extraction and analysis.

Table 3. The Half-life ($t_{1/2}$) of the diazinon in Buffer solutions of pH 6-8

pH	$t_{1/2}$ (Hours)
6	0.074
7	0.022
8	0.077

3.5 The Recovery Studies

For elution, 10 cm^3 of n-hexane was used first to soak the activated charcoal then followed by 10 cm^3 to elute the analyte. This gave an excellent recovery of 75.76-80.68%. this high value might be due to lower interaction of the activated charcoal and matrices in water in relation to diazinon. The percent recovery studies were carried out using SPE and LLE methods and the percentage recovery of diazinon were quite good as shown on Tables 4 and 5 respectively. The percent recoveries of diazinon from DW and DDW using SPE systems and LLE techniques were significantly higher compared to their recoveries from SW using both techniques. However, the percent recovery of diazinon from DDW and DW are somewhat similar. The only exception was SW which showed a small recovery 67.85% using SPE techniques. The difference in the recoveries may be due to the high interaction between matrices in the stream water, the activated charcoal and diazinon for SPE while the LLE involves only the interactions between the matrices in SW and diazinon. Beatriz et al [15] reported a lower percent recovery less than 35% and attributed it to high activity of the activated charcoal and organophosphorus pesticides.

Table 4. Mean percentage recovery and analyte loss of diazinon from water using SPE systems

DW	80.68 + 1.35	23.98
DDW	78.74 + 0.90	27.00
SW	67.85+ 1.28	47.38
Mean	75.76 + 1.18	32.79

Elution was with 10cm^3 n-Hexane. Results are the average of three determinations

Aranda and Kruus [16] reported that, the presence of fulvic and humic acids are ubiquitous in stream water and they interact with organophosphorus insecticides reducing their recovery from 60%-46% during extractions. This may be the reason why the recovery of diazinon from DW and DDW were higher than the recoveries obtained from SW. This also indicates that diazinon have significant interactions with the fulvic/humic acids, clay and silt (matrices) in the stream water. This is in agreement with the study reported in Ohio University Bulletin [17] that, clay, silt hold water and dissolved chemicals longer because of their greater surface area to increase absorption and cannot be easily eluted.

Table 5. Mean percentage recovery and analyte loss of diazinon from water using LLE Technique

DW	96.60 + 1.40	3.52
DDW	93.80 + 1.28	6.60
SW	75.54 + 1.48	32.38
Mean	88.65 + 1.39	14.16

Elution was with 10cm^3 n-Hexane. Results are the average of three determinations

The mean percent recovery of diazinon from the various types of water ranges from 75.76% to 88.65% for both techniques as indicated on table 4 and 5 shows excellent recoveries for SPE and LLE techniques. Sapna et al. [18] reported the mean percent recovery of 98.79% for diazinon from soft drinks using LLE. The low percent recovery for diazinon from this work as compared to the work of Sapna et al. [18] may be due to the use of different extraction solvent and the chemical interaction of other organic substances in the water with diazinon. In this study, there was no attempt made to determine the organic substances present in the stream water.

The lower mean percent recovery from SPE systems as compared to LLE techniques may be due to the adsorption of analytes on to the granulated activated charcoal (GAC) and interaction of the pesticides with the mobile phase. The GAC may have trapped the pesticides and other matrices from water before elution such that even after elution some of the analytes were not eluted due to intra-pesticide diffusion into its interior of the GAC and was not able to be eluted. This agrees with the study reported by Edward et al. [10] where C_{18} and C_8 was used in SPE columns to extract seven agrochemicals in water with a percent recovery of 72-98%, that the agrochemicals were strongly adsorbed to be extracted. Sadek et al. [19],

confirmed that, absorption of phenoxylalkanoic acids on graphitized carbon as a sorbent is assumed to occur initially at the surface of the sorbent particles which may subsequent diffuse into the interior or become covalently bonded (intrapesticdes diffusion or chemosorption respectively) which eluting solvent may not be able to penetrate into the interior and dissolve the analyte and elute completely resulting in tailing. Oscik-Mendyk and Royzylo [20] reported that interaction and molecular associations in the mobile phase can occur resulting in degradation of the analyte and were independent of the nature of the sorbent used. This may be the reason why there was low recovery from stream water. This also, is in agreement with the studies carried out by Sadek et al. [19] and Smith et al. [21] in which they reported that any strong interaction of the analyte with residual hydroxyl sites or complexation with trace metals present in the water can lead to tailing and irreproducitibility of recoveries. They also investigated the influence of trace metal retention of analytes on sorbent and concluded that, they affect retention and elution. David et al. [22] reported that any extraction method developed on spiked samples will not necessary yield high recoveries of spiked analyte when significant analyte interaction exist and does not elute.

3.6 LLE Technique

The LLE technique was used to extract diazinon from various types of water to determine whether there was analyte loss during the extraction and that SPE systems were in control. Soren, et al. [23], affirms that in spiked recovery studies including SPE techniques, LLE technique should be performed to demonstrate collection efficiencies and to demonstrate that no loss of target analyte occur during extraction. The results obtained from this study using LLE technique to extract diazinon from DW, DDW and SW indicates that the percentage analyte loss values for Diazinon extracted from DDW and DW was less than the value obtained in SW (Tables 4 and 5). The higher percent recoveries obtained in SW may be due to the presence of different matrices, and activated charcoal which may interact with diazinon thereby reducing their recovery from the stream water while the lower values of percentage analyte loss may be due to the fact that DDW and DW are significantly free from these matrices. Tables 4 and 5 show an increase in percent analyte loss from SW as compared to DDW and SW for diazinon using both techniques. This may be those diazinons

interact with the activated charcoal and did not elute completely from the GAC solid phase extraction column. The mean analyte loss from extraction using SPE systems were approximately twice that of LLE techniques. Although, similar mean analyte loss were obtained for diazinon using LLE technique as compared to their loss in SPE system. The difference in mean percent loss may be due to diffusion of the analyte into the interior of the GAC and its interaction with water matrices and difficult to be eluted with n-hexane as compared to LLE techniques which has no sorbent but can only interact with water matrices. However, the comparison revealed that there was a significant loss of analyte during SPE using granulated activated charcoal.

4. CONCLUSION

The results obtained in this study show that granulated activated charcoal can be used as an adsorbent in SPE technique to extract diazinon from water samples and eluted with n-hexane. I recommend that, there should be more researches on the usage of SPE systems for extraction of other organophosphorus insecticides using granulated activated charcoal as an absorbent and its number of cycles should also be determined. Similarly, other solvents such as dichloromethane and acetonitrile should be used for elution of diazinon from granulated activated charcoal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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