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Development of Green Method for Trace Determination of Bendiocarb in Real Samples Using Emerson Reaction

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Abstract:

In this study, cloud point extraction combined with molecular spectrometry as an eco-friendly method is used for extraction, enrichment and determination of bendiocarb (BC) insecticide in different complex matrices. The method involved an alkaline hydrolysis of BC followed Emerson reaction in which the resultant phenol is reacted with 4-aminoantipyrene(4-AAP) in the presence of an alkaline oxidant of potassium ferric cyanide to form red colored product which then extracted into micelles of Triton X-114 as a mediated extractant at room temperature. The extracted product in cloud point layer is separated from the aqueous layer by centrifugation for 20 min and dissolved in a minimum amount of a mixture ethanol: water (1:1) followed the determination of BC by using spectrophotometry at a wavelength maximum of 470 nm. The most important parameters affecting the extraction and determination of BC are conducted via a classical optimization. Under the optimum conditions established, Beer's law is obeyed in the range of 0.1-4 $\mu\text{g mL}^{-1}$ while the optimum concentration ranges estimated by Ringbom's plot was of 0.4-2.12 $\mu\text{g mL}^{-1}$. The enrichment factor was of 59.87 fold leading to achieve the limit of detection of 0.076 $\mu\text{g mL}^{-1}$. The proposed method gives superior sensitivity in terms of the molar absorptivity of $1.99 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and extraction efficiency of 98.0%. The established method is applied in the analysis of the spiked vegetables, orange, soil and water samples with appropriate concentration with BC standard.

Key words: Cloud point extraction, UV-Vis Spectrophotometry, Bendiocarb Pesticide, Emerson reaction

Introduction:

Bendiocarb classified as N-methylcarbamate and its IUAPAC named as (2, 2Dimethyl-1, 3-benzodioxol-4-yl) N-methylcarbamate, is a white to light brown powder with

water solubility of 280 mg L^{-1} at 20°C and pH=7. Its chemical structure is displayed in Figure 1[1].

Bendiocarb as an insecticide is widely used in agricultural or residential

purposes because of its importance in boosting crop production

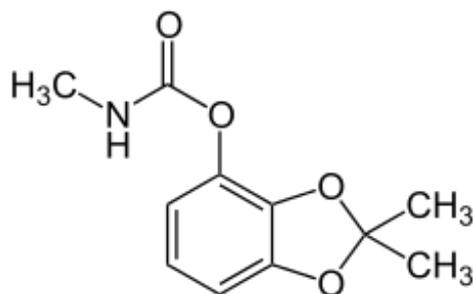


Fig. (1): The chemical structure of Bendiocarb (C₁₁H₁₃NO₄, 223.23 g/mol).

yields, and effective in controlling most diseases caused by harmful insects to humans and animals. As a result of increased use of this insecticide, it inevitably may have undesirable effects which can be up to be toxic ultimately to humans. In the light of this information, risk evaluation of any pesticide requires information on the toxicological properties and environmental toxicity of these compounds as well as their levels in food and environmental components [2]. There is therefore a need for analytical methods to implement reliable monitoring of pesticide residues in those samples. The determination of pesticides in food and environmental matrices is challenge task due to their presence in too low concentrations, which requires the use of efficient method to extract the active ingredient from bulk sample before final assay.

Several methods have been reported for the determination of bendiocarb including gas chromatography (GC) [3-4] high performance liquid chromatography (HPLC) [5-6], gas chromatography-mass spectrometry(GC-MS) [7], liquid chromatography- mass spectrometry (LC-MS) [8], Capillary Zone Electrophoresis (CZE) [9] flow spectrofluorimetry [10], flow injection analysis [11]. Despite the fact that these sophisticated techniques have a high

detection power and sensitivity, but they are relatively expensive and not available in all laboratories. On the other hand, the use of cloud point extraction method in conjunction with molecular spectrophotometric method became an acceptable alternative in pesticide residue analysis due to the numerous advantages, including cheap, easy operated and offers acceptable analytical figures of merit, an integral tool available in most laboratories for the researchers and fulfil the requirement of the green method (i.e. no toxic organic solvent used) [12]. In this regard, a limited number of publications have been appeared in chemical literature using CPE-spectrophotometric method for the determination of pesticides. It is first used by Melchert and Rocha [13] for the determination of carbaryl in natural waters, Santalad et al. [14] described a method for the determination of carbaryl based on acid-induced anionic surfactant micelle-mediated extraction (acid-induced-CPE), and Karnsa-ard et al [15] established CPE combined with visible spectrophotometric method for the determination of carbaryl residue in vegetables.

In this work, CPE-Visible spectrophotometric method is described for the determination of bendiocarb in various samples for the first time. The method is based on an alkaline hydrolysis of BC to phenolic compound which then reacted with 4-aminoantipyrene(4-AAP) in the presence of potassium ferric cyanide as an oxidizing agent to form red colored product according to Emerson reaction, which then extracted into micelles of Triton x-114 as an extracting medium at room temperature. The extracted product in cloud point layer is separated from the aqueous layer by centrifugation for 20 min and dissolved in a minimum amount of a mixture of ethanol: water (1:1) followed the determination of BC

by using spectrophotometry at a wavelength maximum of 470 nm. The new method has been applied for the determination of BC in water, soil, vegetables and orange samples with QuECHERS method was chosen as a sample preparation method.

Materials and Methods:

Apparatus

A recording of absorption spectra and all absorbance measurements of target analyte were performed by using Shimadzu double-beam UV-Vis Spectrophotometer model UV-1800 (Kyoto, Japan) working at a wavelength of 190-1100 nm and equipped with 5-mm optical path cell. A portable pH-meter micro-processor (HANNA, Germany) was used for solution pH checking. Water bath (WNB7-45) Experts BASIC English (D10329) was employed during the course of CPE experiments.

Materials and Regents

Bendiocarb (BC) insecticide (99.0% purity, mol wt. 223.23 g mol⁻¹) was purchased from Accustandard[®] (Connecticut, USA). A standard stock solution 1000 µg mL⁻¹ of bendiocarb was prepared by dissolving 50 mg of insecticide in a mixture of 3 mL ethanol: water (1:1) in a 50 mL volumetric flask and diluted to mark with distilled water and kept in an amber bottle in refrigerator. 4-amino-antipyrene (98% purity, mol. wt. 203.24 g mol⁻¹) was supplied from Himedia Laboratories (India). A 1x10⁻³ M of 4-aminoantipyrene was prepared by dissolving 0.02030 g in 5 mL water and diluted to mark in 100 mL volumetric flask. Potassium ferric cyanide (99% purity, mol. wt. 329.27 g mol⁻¹) was obtained from Sigma-Aldrich (Germany). A 1x10⁻³ M of K₃Fe(CN)₆ was prepared by dissolving 0.03290 g in 5 mL water and diluted to mark in 100 mL volumetric flask. Potassium chloride

from GCC Laboratory Reagent (Gainland Chemical Co., UK). A 0.005 M KCl was prepared by dissolving 0.0372 g in 5 mL water, complete to mark with water in 100 mL volumetric flask. Triton X-114 (purity >99.9%), was purchased from Amresco LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL in 100 mL water. The distilled water was used in the preparation of all solutions and for final rinsing of glass wares.

General CPE Procedure

To an aliquot of 10 mL of a solution containing known amount of bendiocarb standard (0.1-4 ng mL⁻¹) or in sample solution were taken into 10 mL graduated centrifugal tubes; 1.5 mL of 2 M Na₂CO₃ solution was added and kept for 15 min at room temperature for complete BC hydrolysis to phenol. Then, 1.5 mL of 2M HCl was added to neutralize the Na₂CO₃ followed by adding 1 mL of sodium borate/sodium hydroxide buffer solution (pH=9). After that, 0.04 mL of 1x10⁻³ M 4-AAP solution, 0.3 mL of 1x10⁻³ M potassium ferric cyanide, 0.7 mL of 5x10⁻³ M KCl and 0.8 mL of 10%(v/v) Triton X-114 were added and diluted to mark with water. The separation of the phases was accelerated by centrifuging at 3500 rpm for 20 min. After decantation of aqueous phase, the surfactant-rich phase that remained adhered to the tube was dissolved with a 1 mL of ethanol: water (1:1) and the absorbance of each solution containing bendiocarb was measured spectrophotometrically in 5-mm quartz cell at λ_{max} of 470 nm.

Samples Preparation

Water: About one liter of drinking and river water samples was randomly collected from the campus of University of Baghdad / Iraq. The river water was first filtered off to remove any suspended materials and all samples were kept in refrigerator until analyzed. Each sample was spiked with different

concentration of bendiocarb standard solution and subjected to general CPE procedure and BC was determined by spectrophotometry at λ_{\max} of 470 nm.

Soil: The soil sample was randomly collected from home garden and the soil sample solution was prepared according to the procedure adopted by Pourreza et al [16] with little modification. The sample was air-dried at room temperature, grinded in agate mortar into small particle size of about 250 μm sieve and stored in closed vessel. A 20 g of sample was weighted in 100 mL conical flasks and 40 mL of 0.1 M HCl was added. The content was shaken in a mechanical shaker for one hr., then filtered and the pH of the filtered was adjusted to 7.0 by diluted NaOH. Three portions of the resultant solution was directly spiked with different concentrations of bendiocarb standard and subjected to general CPE procedure and target analyte was determined by spectrophotometry at λ_{\max} of 470 nm.

Vegetable and orange samples: The vegetables (Cucumber and Tomato) and orange were purchased from local markets in Baghdad, Iraq. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method used for pesticide residue analysis was adopted with little modification [17] for sample preparation of vegetables and orange. A 0.5 kg of each sample was selected and the edible part was cut into 1-cm pieces and blended using a commercial food mixer for homogenization the sample. A 15 g sample portion was placed in 100 mL conical flask and 20 mL of solvent mixture containing 1:5 acetic acid and acetonitrile was added and the content was shaken vigorously in an electrical shaker for one hr. After shaking, the extract was withdrawn and transferred into 50 mL centrifugal tube and mixed with 10 g sodium sulphate, 4 g magnesium sulphate and 1 g of sodium

acetate and centrifuged for 10 min at 250 rpm to separate the phases. The upper layer was taken and mixed with 0.3 mL weak anion exchanger and 0.6 g carbon black in another centrifugal tube and immediately shaken and filtered. The filtrate solution was evaporated at 50 °C on water bath to remove the solvent. The residue was dissolved with water then diluted to 10 mL in standard volumetric flask. Each sample solution was spiked with different concentration of bendiocarb standard and subjected to general CPE procedure and target analyte was determined by spectrophotometry at λ_{\max} of 470 nm.

Statistical Analysis:

Excel 2007 (Microsoft Office®) and Minitab version 14(Minitab Inc., State College, PA, USA) were employed to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

Results and Discussion:

Absorption Spectra

Several published reports have confirmed that the reaction between phenols with 4-aminoantipyrene (4-AAP) in the presence of mild oxidizing agent forms colored dye [18-20]. Thus preliminary tests were made without using CPE to ensure the formation of the colored product between alkaline hydrolyzed bendiocarb and 4-AAP in presence of potassium ferric cyanide at concentrations of 1×10^{-3} M, and indeed a red water soluble product was formed. Figure 2 shows that the visible absorption spectrum (d) of the coupled product in the surfactant-rich phase at optimum conditions exhibits a maximum absorption at 470 nm against the blank solution, and therefore all absorbance measurements were performed at this wavelength throughout this study. Whilst other individual reaction constituents such as bendiocarb,

4-AAP and potassium ferric cyanide solutions have shown different absorption maxima of (a) 273 nm, (b) 372 nm, and (c) 260, 302 and 420 nm respectively as displayed in Figure 2.

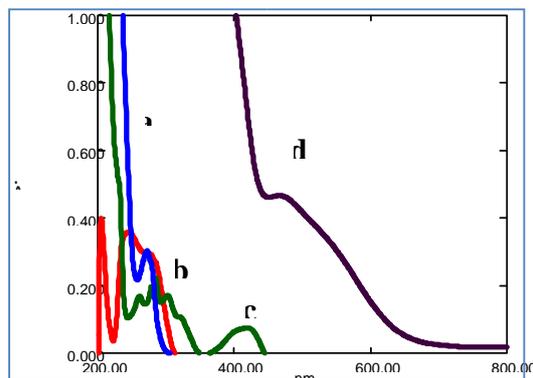


Fig. (2): Absorption spectra
Absorption spectra (a) 4.47×10^{-6} M Bendiocarb solution (b) 5×10^{-5} M 4-aminoantipyrene solution and (c) 5×10^{-5} M potassium ferric cyanide solution (d) Bendiocarb-4-aminoantipyrene coupling product by general CPE procedure

Alkaline hydrolysis of Bendiocarb

It was reported that all carbamate pesticides are easily hydrolyzed in alkaline medium to form phenols [21-22]. Consequently, different alkaline media were preliminary examined at different concentrations (0.05-0.6 M) such as NaOH, KOH, NH_3 and Na_2CO_3 to choose the best one that give the phenolic product of $0.1 \mu\text{g mL}^{-1}$ bendiocarb at λ_{max} of 279 nm. It can be seen (Figure not shown) that Na_2CO_3 at concentration of 0.3 M gave the best sensitivity compared with other alkaline media at 5 min hydrolysis. Also, the effect of hydrolysis time was studied by varying time between 0 and 45 min. It was found (results not shown) that 15 min was good enough for complete hydrolysis of insecticide [23]. Therefore, 0.3 M Na_2CO_3 and 15 min were selected for subsequent CPE experiments.

Optimization of CPE Conditions

A preliminary observation indicated that the coupling dye product is hydrophobic and can be easily extracted in non-ionic surfactant. Hence, investigation of the reaction product was based on of Triton X-114 as an extracting medium at room temperature. Therefore, the studies were directed towards optimizing the experimental CPE conditions in order to maximize the sensitivity of the method and the extraction efficiency of the target analyte. The parameters such as pH, 4-AAP concentration, oxidizing reagent concentration, KCl concentration, Triton X-144 amount, and order of additions were conducted using a classical optimization strategy followed the general CPE procedure.

The effect of pH

The pH is an important variable for quantitative reaction between BC and 4-AAP in the presence of potassium ferric cyanide keep of the colour derivative product in alkaline solution to prevent its decomposition [18, 24], and subsequent extraction in the micelle-mediating solvent. The effect of pH was performed by taking 10 mL solution containing $1.0 \mu\text{g mL}^{-1}$ of bendiocarb in a series of 10 mL centrifugal tubes, 1.5 mL of 2 M Na_2CO_3 was added and kept the solution stand with occasional shaking for 15 min. After the counteract the alkalinity with dilute HCl, 1 mL of 1×10^{-3} M of 4-AAP, 1 mL of 1×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$, 1 ml of 5×10^{-3} M KCl and 1 mL of 10% TX-114 were added with varying pH from 7-11 using sodium borate-NaOH buffer solution and followed the general CPE procedure. The absorbance of each solution was measured at λ_{max} of 470 nm as displayed in Figure 3.

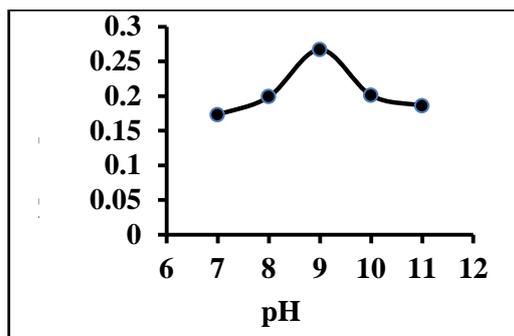


Fig.(3) :The effect of pH on the absorbance of derivative product. CPE conditions: equilibration 25 °C

It can be seen that the absorbance increased gradually up to pH=9 and then suddenly decreased after pH=10. Thus, pH of 9 was chosen as optimal for subsequent experiments.

The effect of 4-AAP Concentration

The effect of different concentrations of 4-AAP on the absorption signals of 10 mL solution containing $1.0 \mu\text{g mL}^{-1}$ of BC at pH=9 was studied according to the general CPE procedure by adding successive increase of 1×10^{-3} M of 4-AAP with varying volume in the range of 0.01-0.1 mL, while keeping other parameters mentioned in the above section constant. It was shown (Figure 4) that the maximum absorbance measured at λ_{max} of 470 nm was optimum at 4×10^{-6} M of 4-AAP. Beyond this concentration, any excessive amount of reagent was not necessary. Therefore, 0.04 mL of 1×10^{-3} M was used for further experiments.

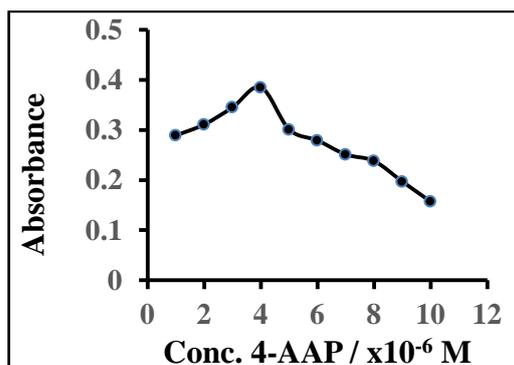


Fig.(4): The effect of 4-AAP on the absorbance of derivative product by CPE

The effect of $\text{K}_3\text{Fe}(\text{CN})_6$ concentration

The impact of $\text{K}_3\text{Fe}(\text{CN})_6$ as an oxidizing agent plays an important role in the stage of coupling reaction between the hydrolyzed BC and 4-AAP. Therefore, the effect of various concentration of $\text{K}_3\text{Fe}(\text{CN})_6$ was studied on the absorbance of 10 mL solution containing $1 \mu\text{g mL}^{-1}$ of bendiocarb according to the recommended CPE procedure by varying the volume in the range of 0.1-1.0 mL of 1×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$ and keeping the previous optimum parameters constant. The results are shown in Figure 5. It was noticed (Figure 5) that the absorbance signals increased with increasing the concentration of $\text{K}_3\text{Fe}(\text{CN})_6$ solution up to 3×10^{-5} M and after this concentration the coupling reaction was not significantly influenced. Consequently, a 0.3 mL of 1×10^{-3} M of reagent was selected as optimal.

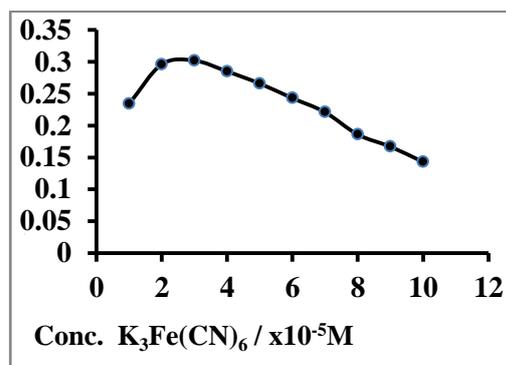


Fig.(5): The effect of $\text{K}_3\text{Fe}(\text{CN})_6$ on the absorbance of derivative product by CPE

The effect of KCl Amount

The addition of some inorganic salts (i.e. an electrolyte) can facilitate phase separation and improve extraction efficiency due to the salting out of cations that are present in the aqueous micellar solution due to altering the density of the bulk aqueous solution thus leading to lower the cloud point temperature of the surfactants [25-26]. In this regard, the effect of KCl amount was studied on the extraction of 10 mL

solution containing $1 \mu\text{g mL}^{-1}$ of BC according to the recommended CPE procedure by varying the volume in the range of 0.1-1.0 mL of 5×10^{-3} M KCl and keeping the previous optimum parameters constant. The results are shown in Figure 6. The findings revealed that KCl at concentration of 3.5×10^{-4} M provided a high absorbance and therefore chosen as optimal.

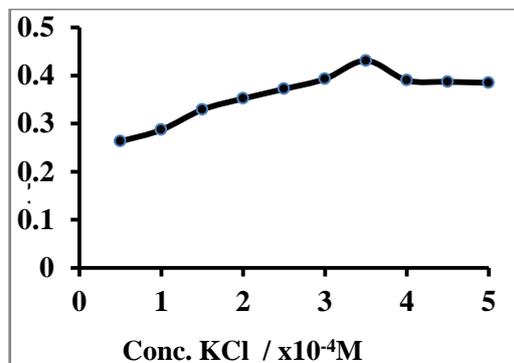


Fig. (6): The effect of KCl the absorbance of derivative product by CPE.

The effect of TX-114

The amount of surfactant as an extracting medium is very important in order to achieve maximum extraction efficiency by minimizing the phase volume ratio (V_s/V_a) and therefore improving the preconcentration factor of the method [27]. The variation of absorption signal on the formation of coupling product with Triton X-114 amount was tested within the surfactant volume range of 0.1 – 1.0 mL of 10% (v/v) Triton X-114, keeping other optimized parameters constant. As can be seen from in Figure7, the absorbance of the colored product increased with increases Triton X-114 amount up to 0.8 mL of 10% (v/v) and then slowly decreased at higher amounts. Therefore, a 0.8 mL of 10% (v/v) Triton X-114 was used as the optimum amount to obtain a high pre-concentration factor in the following experiments.

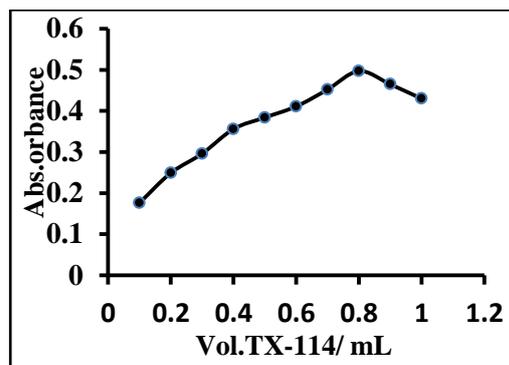


Fig.(7): The influence of Triton X-114 on the absorbance of derivative product by CPE

The effect of Order of Additions

The effect of order of addition on the absorption signal of the Red derivative product was also examined. Table 1 reveals that the best order of addition is number 2 due to give a highest absorption signal among the others.

Table (1): The effect of order of addition

No.	Order of addition	Abs.
1	BC +KCl+ $\text{K}_3\text{Fe}(\text{CN})_6$ +4-AAP +TX-114	0.354
2	BC + 4-AAP + $\text{K}_3\text{Fe}(\text{CN})_6$ +KCl +TX-114	0.497
3	BC +KCl + 4-AAP + $\text{K}_3\text{Fe}(\text{CN})_6$ + TX-114	0.219
4	BC + $\text{K}_3\text{Fe}(\text{CN})_6$ + 4-AAP+ KCl+TX-114	0.185

Stoichiometry of the Extracted Colored Product

The slope analysis method was used for the determination of the stoichiometric amount between the reaction of bendiocarb and 4-AAP reagent via the analysis of the dependence $\log D = f(\log C_{\text{Reagent}})$ measured under the optimum conditions [28] and using the order of addition No. 2. . Figure 8 shows the slope for $\log D$ as a function of $\log [4\text{-AAP}]$ is equal to 1.2846, indicating the reaction between the BC insecticide and 4-AAP in the coupling reaction to form the colored product with ratio of 1:1 is extracted into the surfactant-rich phase. Thus the most probable reaction is preceded into two steps to form the red product as shown in the Figure 9. The observations were revealed that colored product was

stable for 20 min in surfactant-rich phase as shown in Figure 10.

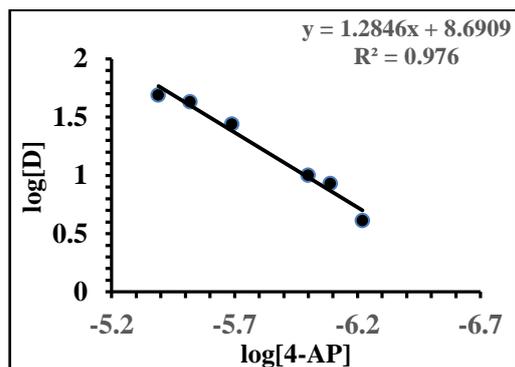


Fig.(8): Slope analysis method for the determination the composition of coupling product

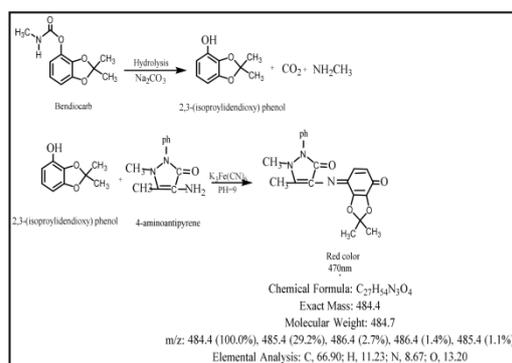


Fig. (9): The most probable coupling reaction path

Analytical figures of merit

A linear calibration curve for the spectrophotometric detection of BC

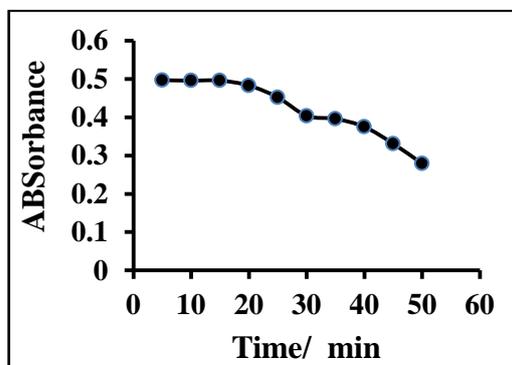


Fig. (10): Variation of absorbance with time for the colored product

was constructed in the range of 0.1–4 $\mu\text{g mL}^{-1}$ standard solution at optimum conditions as shown in Figure 11.

Above $> 4 \mu\text{g mL}^{-1}$ (not shown in Figure), the Beer's law was not obeyed giving a negative curvature toward the concentration axis. This may be due to insufficient reagents to comply with the stoichiometric amount to produce the colored product that absorbs at λ_{max} of 470 nm or most probably the formation of other species which compete with coupling product. Also, Ringbom plot was constructed between $\log [BC]$ and $(1-T)$, where T is transmittance, in order to know the effective optimum concentration range as showed in Figure 12. The plot has sigmoid shape with a linear segment at intermediate absorbance values (0.182– 0.954) and concentration values (0.4–2.12. ng mL^{-1}). This range achieves the greatest photometric accuracy because the measurements should be within 15%–65% T or 0.19–0.82 A. However, the best fit was obtained for a first order equation (Table 2 and Figure 11) with correlation coefficient of 0.9998 and coefficient of determination (R^2) was 99.96% which suggests statistically valid fit. This was supported by ANOVA analysis (Table 3) giving that $MS_{\text{reg}}/MS_{\text{error}} = 18695.43$ of the target analyte for 1 and 6 dof, larger than critical value ($F^1_6 = 5.99$ at 95% CI). We use this fitted linear calibration model to estimate the Bendiocarb concentration in all analyzed samples which appears justified, on statistical basis.

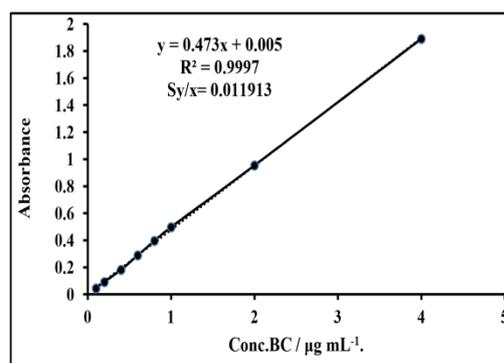


Fig.(11): Calibration curve for BC by the proposed method

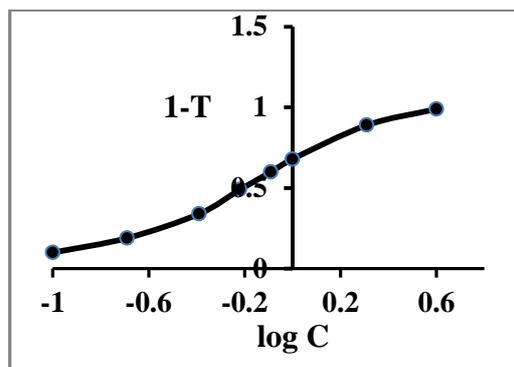


Fig.(12): Ringbom plot of the product by the proposed method

As can be seen that a good enrichment factor of 59.87 fold was obtained (Table 2). This allows achieving limit of detection (LOD) of $0.076 \mu\text{g mL}^{-1}$ and limit of quantitation (LOQ) of $0.252 \mu\text{g mL}^{-1}$ for bendiocarb by developed CPE-Spectrophotometry. This in turn led to obtain a high sensitivity in terms of molar absorptivity which found to be of $1.99 \times 10^5 \text{ L.mol}^{-1} \cdot \text{cm}^{-1}$. Regarding the detection limit, our finding was better than that obtained by other workers such as Dhahir et al [29] who used diazotized coupling of bendiocarb with p-amino phenol, Alvarez-Rodríguez et al [30] used a coupling with diazotized trimethylaniline (TMA) in a sodium dodecyl sulphate and Kumar and Rekha [31] used three different azo dyes coupling reagents, by spectrophotometric detection without CPE, which were of $0.265 \mu\text{g mL}^{-1}$, $0.2\text{-}2.0 \mu\text{g mL}^{-1}$ and $0.285\text{-}0.564 \mu\text{g mL}^{-1}$ respectively. But, it was about much worse than that obtained by Faber and Scholer [4] whom employed a gas chromatography after flash heater methylation with trimethylsulfonium hydroxide, and by Lin et al [9] who used CZE with pre-column hydrolysis and amperometric detection. However, the LOD of the method was also calculated and found to be 0.051 mg kg^{-1} for bendiocarb, by considering a limit of detection of 0.076 mg L^{-1} in aqueous solution and 15 g of vegetable and fruit samples in 10 mL

solution. This finding has encouraged us to apply the proposed method in the estimation of bendiocarb in real samples such as vegetables, fruits and environmental samples to test its applicability and reliability. In fact, the developed method in this work may achieve the requirements of the international standards in terms of the maximum residue limits (MRL) of bendiocarb insecticide in different types of foods set by FAO/WHO that is in the range of $0.1\text{-}0.05 \text{ mg kg}^{-1}$ [32].

Accuracy Test

Since the certified reference materials (CRM's) that specify the exact quantity of bendiocarb are unavailable, the accuracy of the proposed method was accomplished by assessing the recovery percentage by the spiking river and soil samples with 0.4, 0.8 and $2.0 \mu\text{g mL}^{-1}$ standard bendiocarb followed the recommended CPE procedure. The results are presented in Table 4. It can be seen that a good accuracy in terms of recovery percentage can be achieved between 97.25% and 100.2%. This confirmed that the systematic errors are almost absent, concluding the presence of matrix constituents of these sample have no effect on the determination of

Table (2):The statistical data and analytical figures of merits for BC using by CPE- Spectrophotometry

Parameter	Value
Product colour	Red
λ_{max} (nm)	470
Regression equation (11 points)	$y = 0.4730x + 0.0049$
Standard deviation of regression line (Sy/x)	0.011913
Correlation coefficient(r)	0.9998
Coefficient of determination (R ²)	99.96
C.L. for the slope ($b \pm t_{sb}$) at 95%	0.4730 ± 0.00299
C.L. for the intercept ($a \pm t_{sa}$) at 95%	0.0049 ± 0.00498
Beer's law range ($\mu\text{g mL}^{-1}$)	0.1-4
Ringbom plot ($\mu\text{g mL}^{-1}$)	0.4-2.12
Limit of Detection ($\mu\text{g mL}^{-1}$)	0.076
Limit of Quantitation ($\mu\text{g mL}^{-1}$)	0.252
Sandell's sensitivity ($\mu\text{g cm}^{-2}$) $\times 10^{-3}$	0.00212
Molar absorptivity ($\text{L.mol}^{-1} \cdot \text{cm}^{-1}$)	1.99×10^5
Composition of complex*	1:1
RSD% (n=5) at $0.4 \mu\text{g mL}^{-1}$	1.18
RSD% (n=5) at $2 \mu\text{g mL}^{-1}$	0.17
Preconcentration factor	50
Enrichment factor	59.87
Distribution ratio(D)	49
Extraction efficiency(%E)	98

Table(3): ANOVA Table for the regression line by the proposed method

	d f	SS	MS	F	Significance F
Regression	1	2.653295	2.653295	18695.43	1.03E-11
Residual	6	0.000852	0.000142		
Total	7	2.654147			

bendiocarb Meanwhile, each spiked sample was repeated five times for precision testing in term of %RSD and found to be in the range of 0.11-1.31 , indicative a good precision.

Table (4): The accuracy and precision of the proposed method for the determination of Bendiocarb

sample	Amount Bendiocarb taken (ng mL ⁻¹)	Amount Bendiocarb found (ng mL ⁻¹)	Recovery (%)	Erel (%)	RSD (%) (n=5)
River water	0.4	0.391	97.75	-2.25	1.31
	0.8	0.793	99.12	-0.87	1.26
	2.0	1.966	98.30	-1.80	1.06
Soil	0.4	0.389	97.25	-2.75	1.08
	0.8	0.786	98.25	-2.12	0.51
	2.0	2.004	100.2	0.10	0.11

Interferences study

In order to study the selectivity of the proposed method on the determination of bendiocarb , the effect of several foreign species expected being in the selected analyzed samples was studied by addition of 10, 25, 100 µg of each interfering species to the standard solution containing 1 µg mL⁻¹ of BC followed the recommended CPE procedure. The results are presented in Table 5. It is agreed that an extraneous species deemed to interfere seriously when it gives a relative error percent more than ±10% [33]. It can be seen that there is no appreciable effect of most foreign species in the determination of bendiocarb (%E_{rel} less than ±5%), except of vitamin C which caused some interferences thus giving low percent recovery of the colored product. Therefore, this interfering species should be removed or masked before determination of bendiocarb, or added to standard bendiocarb solutions before the construction of the calibration curve.

Table(5): The effect of divers species on the percent recovery of BC by the proposed method

Foreign species	Recovery %			Mean Rec %± Sd
	10 µg	25 µg	100 µg	
Ca ²⁺	96.5	98.8	100.2	98.5±1.86
Mg ²⁺	98.6	100.8	101.4	100.2±1.47
Fe ²⁺	100.1	101.8	99.7	100.5±1.11
Co ²⁺	99.5	102.6	100.4	100.8±1.59
Vitamin B	101.4	100.8	104.1	102.1±1.75
Vitamin C	85.3	90.5	93.7	89.8±4.23
Glucose	98.7	99.4	101.9	100.0±1.68
Fructose	95.8	98.1	100.8	98.2±2.50
Protein	96.3	100.5	102.4	99.7±3.12

Application In an attempt by the initial tests, it shows that all selected samples in this study do not have any residue of BC pesticide. Consequently, all the selected samples in this study were spiked with BC standard at concentration of 0.4, 0.6 and 3 $\mu\text{g mL}^{-1}$ of BC standard solution and subjected to the general CPE procedure for five

replicates measurements and BC concentration in each spiked sample was measured spectrophotometrically at λ_{max} of 470 nm. The results are summarized in Table 6. It was observed that the average recoveries of the spiked BC standard were of 95.8-105.0 % with average RSDs of 0.14- 2.86 %.

Table(6): Analytical results of bendiocarb in different samples by proposed method

sample	Bendiocarb added ($\mu\text{g mL}^{-1}$)	Bendiocarb found ($\mu\text{g mL}^{-1}\pm\text{SD}$)	Recovery% (mean \pm SD)	RSD% n=5
River water	0.2	0.198 \pm 0.05	99.0 \pm 0.95	2.53
	0.6	0.60 \pm 0.15	100.2 \pm 0.61	2.14
	3.0	3.10 \pm 0.03	103.3 \pm 0.38	1.86
Tap water	0.2	0.194 \pm 0.21	97.0 \pm 1.29	1.94
	0.6	0.58 \pm 0.11	96.6 \pm 0.88	1.51
	3.0	2.88 \pm 0.18	96.0 \pm 0.77	0.81
Soil	0.2	0.20 \pm 0.17	100.0 \pm 0.59	1.84
	0.6	0.58 \pm 0.08	96.7 \pm 0.17	1.62
	3.0	2.87 \pm 0.05	95.8 \pm 0.13	0.14
Cucumber	0.2	0.197 \pm 0.14	98.5 \pm 2.26	2.22
	0.6	0.591 \pm 0.23	98.5 \pm 1.79	1.71
	3.0	3.01 \pm 0.28	100.3 \pm 1.28	1.28
Tomato	0.2	0.196 \pm 0.04	98.0 \pm 2.09	1.62
	0.6	0.594 \pm 0.21	99.0 \pm 1.83	1.39
	3.0	2.89 \pm 0.07	96.3 \pm 1.11	1.16
Orange	0.2	0.21 \pm 0.19	105.0 \pm 3.00	2.86
	0.6	0.59 \pm 0.24	98.3 \pm 2.50	2.55
	3.0	3.05 \pm 0.09	101.7 \pm 1.90	1.87

Conclusions:

Emerson Reaction has been exploited in the development of environmentally-friendly method based on CPE combined with visible spectrophotometry for the determination of bendiocarb insecticide in various samples. The established method provides the substantial analytical figures of merits which were better than that obtained with those reported methods published in chemical literatures. The solubilization of the resultant colored product in micelle occurs at room temperature, thereby the steps of equilibration temperature and incubation time has been ruled out from CPE procedure and this is in itself a great benefit in terms of economy of time and effort. The proposed method can be considered as an alternative to

the other sophisticated techniques such as LC-MS, GC-MS and electrophoresis.

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تطوير طريقة صديقة للبيئة لتقدير الاثر لمبيد البينديوكارب في عينات حقيقية باستعمال تفاعل ايمرسون

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الخلاصة:

في هذه الدراسة، تم استعمال تقنية الاستخلاص بنقطة الغيمة مع المطيافية الجزيئية بوصفها طريقة صديقة للبيئة لاستخلاص واغناء وتقدير مبيد البينديوكارب في مناشئ معقدة مختلفة. اعتمدت هذه الطريقة في بادئ الامر على التحلل القلوي للمبيد ثم اعقب ذلك تطبيق تفاعل ايمرسون الذي يتفاعل الفينول الناتج مع 4-امينوانتبارين بوجود بوتاسيوم سيانيد الحديد كعامل مؤكسد قلوي لتكوين ناتج احمر اللون الذي يستخلص في مايسيلات التريتون $\times 114$ كوسيط استخلاص عند درجة حرارة الغرفة. بعدها يفصل الناتج المستخلص من طبقة نقطة الغيمة بالطرد العمركزي لمدة 20 دق ومن ثم يذاب في كمية قليلة من مزيج الايتانول: الماء (1:1) لغرض تقدير المبيد طيفيا عند الطول الموجي الاقصى 470 نانوميتر. كما تم دراسة المتغيرات المهمة التي تؤثر على استخلاص وتعيين المبيد باستعمال اسلوب الامثلية التقليدية، فضلا عن دراسة التداخلات للتحقق من ضبط الطريقة. وتحت الظروف الفضلى المتبعة، تبين ان قانون بير اطاع في مدى تركيز 1.0-4 مايكروغرام مل⁻¹، في حين اعطى الرسم البياني لرنكبوم المدى الامثل لمنحني المعايرة وكان بحدود 4.0-12.2 مايكروغرام مل⁻¹. كما اظهرت النتائج ان عامل الاغناء كان بمقدار 87.59 مرة مما ادى الى الحصول على حد كشف بمقدار 076.0 مايكروغرام مل⁻¹ وامتصاصية مولارية ممتازة وبمقدار $(10^5 \times 1.99)$ لتر مول⁻¹ سم⁻¹ وكفاية استخلاص بمقدار 98%. تم تطبيق الطريقة المستحدثة في تعيين البينديوكارب في عينات الخضروات والبرتقال، والتراب والمياه المدعمة بتركيز معينة من محلول المبيد القياسي.

الكلمات المفتاحية: الاستخلاص بنقطة الغيمة، المطيافية الجزيئية، مبيد البينديوكارب، تفاعل ايمرسون